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A SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE

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For this Volume Dr. J. A. Arkwright, F.R.S., Dr. A. D. Gardner, Professor R. T. Hewlett and Dr. H. Schütze have assisted in the editorship with regard to particular sections.

A SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE

VOLUME IV

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CHAPTER I. *BACILLUS TYPHOSUS.*

By D. Harvey (Royal Army Medical College).

With Sections by H. M. Perry and H. J. Bensted (Cairo), and W. Bulloch (London Hospital).

History.

By W. Bulloch.

Up to the end of the eighteenth century great confusion prevailed with regard to the fevers indiscriminately called putrid, adynamic, mucous, ataxic, typhus or gastro-enteric. In the first years of the nineteenth century a special type was disentangled from this congeries, mainly as the result of pathological anatomical studies in Paris. Thus P. A. Prost (1804) first drew attention to the fact that the invariable anatomical seat of mucous fever is the wall of the intestine. Petit and Serres (1813) were more exact in locating what they termed ' fièvre-entéro-mésentérique ' to the lower part of the ileum. It is, however, to P. F. Bretonneau, the famous clinician of Tours, that we owe the exact knowledge of the nature and lesions of the disease. It was apparently in 1819 that he convinced himself that the lesions were almost confined to the lymphatic apparatus of the small intestine and particularly to the patches of Peyer. His teaching was disseminated by his pupils. During his lifetime Bretonneau (1829) himself published only one paper on the subject, but in it he maintained that the disease—which he named ' Dothinéntérie ' was contagious. It was accompanied by a specific intestinal eruption, but it was not caused by the eruption. He showed also that one attack of the disease conferred protection. Bretonneau's full account of dothinéntérie was unearthed by Dubreuil-Chambardel and was published by him in 1922, a century after its composition. Louis (1829) gave the disease the name ' fièvre typhoide ', a name that was at once generally adopted.

In England, the French doctrines were only accepted late and especially through the influence of W. Jenner (1850), W. Budd (1859, 1860), and C. Murchison (1862).

The first tangible results in the bacteriology of typhoid were reported by Eberth (1880). He examined 23 cases and found bacilli in the spleen 12 times and in the mesenteric glands 6 times. In 1881, Eberth examined a further series of 17 cases and again found bacilli in 6, but there were no such bacilli in 26 cases of diseases other than typhoid fever. He was of opinion that the ' typhoid bacilli ' stood in some relation to the morbid process. Koch (1881) saw and photographed bacilli in the kidney, spleen

and liver in cases of typhoid. It was, however, Gaffky (1884) who first successfully applied the new cultural methods of Koch to the problem of typhoid fever and obtained from the spleens 25 pure growths of the micro-organism we now know to be the cause of the disease. The bacillus was motile, and unable to liquefy gelatin. On sterile potato a characteristic effect was produced. To the naked eye there was no suggestion that a growth had developed, but microscopic examination revealed the fact that the whole surface of the medium was covered with the bacilli. On potatoes at 37° C. Gaffky found spores, but none in gelatin at room temperature. This was perhaps the only error in his research and was probably due to some sporing contaminant itself. Gaffky failed to find the bacilli in the stools or in the blood. He injected pure cultures into 60 animals and birds and in no single instance was he successful in producing disease. Notwithstanding, he considered that the bacilli were probably the cause of typhoid fever, and he discussed the whole problem of the ætiology, mode of infection and prophylaxis on this basis, in a manner which has made his paper a classic in bacteriological literature. In general, his results were confirmed by Seitz (1886), E. Fraenkel and Simmonds (1885, 1886), Rietsch (1886) and many others.

The nature of the immunity to experimental infection with typhoid bacilli was studied by Beumer and Peiper (1887) and R. Stern (1892), and in a complete manner by R. Pfeiffer and Kolle (1896[2]).

The discovery of the agglutinating action of typhoid serum and its application to the clinical diagnosis of typhoid fever occurred in 1896, and was the work of Durham (1896), Gruber (1896), Widal (1896), and Grünbaum (1896). A good deal of acrimonious discussion has taken place with regard to the respective merits of these workers, but there is little doubt now that the pioneer was Max Gruber, with whom H. E. Durham worked in friendly collaboration in Vienna. The first actual publication with admitted acknowledgment to Gruber was by H. E. Durham (1896, Jan. 3), and in rapid succession, Gruber and Durham (1896[1], March 12, and 1896[2], March 31). Already in Durham's first publication (1896) attention was drawn to the possibilities of the method for the clinical diagnosis of typhoid. F. Widal did this (1896, June 26), and shortly after, A. S. Grünbaum (1896, Sept. 19). There is a tendency to avoid the name of any individual in association with this test.

The active immunization of man against typhoid fever developed out of the results obtained by Haffkine with his cholera vaccine in India, and was first published by A. E. Wright (1896, Sept. 19). In this paper he described the effect of inoculating a dead culture of typhoid bacilli into two medical officers of the Indian Medical Service. The first of these, designated ' M.D.' (Maxwell Dick), was first inoculated with $\frac{1}{20}$ of a tube of killed typhoid bacilli on July 31, 1896. He had two other inoculations on Aug. 14 and Sept. 5, and finally a dose of living typhoid bacilli on Sept. 25 (Wright and Semple, 1897). A. E. Wright informs me (March 27, 1928) that no typhoid fever resulted from the living culture. R. Pfeiffer

and Kolle (1896[1], Nov. 12) also immunized human beings with cultures of typhoid bacilli, but the subsequent history of typhoid inoculation and its wide dissemination has been associated with the name of A. E. Wright.

From the beginning of the present century an intensive typhoid campaign was inaugurated in Germany, chiefly through the suggestions of R. Koch in a famous address at the Kaiser-Wilhelms-Akademie, on Nov. 28, 1902 (Koch, 1903). On his recommendation a number of bacteriological stations were instituted in South-West Germany for the purpose of testing his opinion that the chief source of typhoid infection is to be found in man himself. A large number of important facts accrued from this study, not only in Germany, but also all over the world. Prominent among these facts was the discovery of the typhoid carrier state, which was first put on a bacteriological basis by von Drigalski (1904), and following him by many others, a full account of whose work will be found in the publication of Ledingham (1910). The Great War in all its theatres gave opportunities on an unprecedented scale for applying all the accumulated knowledge on typhoid fever and resulted in many publications.

W. B.

Definition of Bacillus typhosus (Synonyms: Bacillus of Eberth; Eberthella typhi).

B. typhosus is a Gram-negative, non-spore-forming, actively motile bacillus. It forms translucent, irregular colonies on gelatin media and a faint, nearly colourless growth on potato; grows best in the presence of oxygen, but can grow in its absence. It produces acid but no gas in maltose, glucose and mannitol, but does not ferment lactose or saccharose. It produces a slight initial acidity in litmus milk, the reaction after two weeks becoming either neutral or slightly alkaline. It does not form indole or liquefy gelatin, does not reduce neutral red, but causes browning of lead acetate. It is easily killed by acids, but has a high tolerance for brilliant green and alkalis. It has characteristic serum reactions, and is found in human stools and urines as an actual or potential cause of typhoid fever.

Morphology and Staining Reactions.

Form. It is difficult to demonstrate the bacillus in the tissues, but colonies may be seen in sections made from the spleen of a case dead of the fever. Individual members of these colonies can be made out as small bacilli with oval ends, 2 to 4μ in length and about 0·5μ in breadth. The bacilli can also be seen in sections made from non-ulcerated Peyer's patches, and in this material they have the same appearance; but here it is possible that they may be confused with other intestinal bacilli, e.g. *B. coli*.

In culture the form of the bacillus varies according to the media employed. If removed from the dryer upper surface of an agar slope and

examined either in a fresh preparation as a hanging-drop specimen, or fixed on the slide and stained, they will be found to be much as described in the tissues—that is, small bacilli with oval ends and about 2 to 4μ in length and 0·5 to 0·7μ in breadth ; but if the specimen is taken from the water of condensation of the agar culture or from a broth culture it will show longer forms, some very long, like those described later from the urine of a carrier.

Motility. All these forms, including the filaments, can be shown with appropriate methods to be possessed of true motility : some of the small forms can be seen to dart clear across the field of the microscope. The movement of the filament is more slow and wavy.

Flagella. This motility is due, as can be demonstrated by appropriate staining methods, to the possession of peritrichous flagella—long wavy flagella attached along the sides and at either end or occasionally bunched at one end. These may be from 8 to 14 in number and 6 to 8μ in length.

Spores. No method of staining has succeeded in demonstrating spores, but some observers state that certain unstained portions seen in some bacilli are of this nature, and that bacteria showing this condition are more resistant to heat and other destructive agencies.

Capsules. True capsules have not been demonstrated in culture by the ordinary stains used for this purpose, but occasionally in the tissues a halo may be made out around some of the bacilli. Hort (1920) described capsulated forms with a double contour on glucose agar ; but these are not true capsules.

Staining. The bacilli, both in tissues and in culture, are very readily decolorized by Gram's method. They take up other basic stains well, and excellent methods of demonstrating them in tissues or from cultures are simple staining with carbol thionin, or, in sections, Leishman's stain.

Urine. In the urine of a carrier the bacilli are much longer than in the tissues, and filaments may be seen which, if examined in a hanging-drop preparation, may show undulating motility. These may be of a length of 30μ and from 1 to 1·5μ in breadth. Small ordinary forms can also be made out, measuring 5μ in length and 0·7μ in breadth ; these also are actively motile.

Blood. At one time it was stated that the finding of Gram-negative bacilli in the blood was a possible method of diagnosis of typhoid fever. But it must be remembered that to obtain a successful culture it is, as a rule, necessary to take at least 5 c.cm. of blood. It is obvious, therefore, that the chance of discovering a bacillus in a drop of blood is remote.

Variations—Involution, Pleomorphism, Branching.

It has been noted that if a very careful examination of several films is made from an ordinary culture of *B. typhosus*, one or two bacilli may be seen which appear as if they were contaminations. These are much larger, broader and more deeply stained than the usual type, and they may measure 10 by 2μ. A great deal has been written of recent years regarding

these aberrant forms, and some writers have attempted to base far-reaching conclusions as to the reproduction and life-history of the bacillus on the evidence of such forms.

Walker and Murray (1904), whilst studying intra-vitam staining, found that if various dyes, e.g. aniline methyl violet, 2 per cent., were introduced into culture medium and the culture incubated at 40° C., *B. typhosus* might show true branching forms on solid media. Long filaments also appeared with sinuous movements, and in older cultures segmentation could be made out, and later on an appearance as of a chain of cocci. Giant forms were noted stretching across the field of the microscope, which were much broader than the ordinary forms, but they possessed flagella and were agglutinated by specific antisera.

Hort (1920), using media with 4 per cent. glucose, demonstrated somewhat similar forms. He attributed their occurrence to the fact that they were growing under ' optimum ' conditions, and held that, therefore, they could not be termed involutions forms, as they appeared in the course of a few hours ; but, as a matter of fact, when the bacilli grow in a medium with so high a percentage of glucose, acid is very rapidly formed, and in such quantity as first to inhibit growth and ultimately to destroy the culture. It is, therefore, probable that the appearance of these forms is due in part to the high degree of acidity of the medium.

Hort also described and demonstrated various curious forms in a pure culture of *B. typhosus* in 4 per cent. glucose media. Some were thick-walled, which he called ' double contour ' forms. These were *non-agglutinable*, or at least showed only fine granular agglutination ; also giant forms, deeply staining, branching forms, and small coccal forms which were non-motile were demonstrated. The majority were ' fertile '—that is, they could be seen to divide by transverse fission. The resulting individuals were often not equal in size ; it is, therefore, not correct to say, as is usual, that the invariable method of reproduction in *B. typhosus* is by equal binary fission. Some curious spirochætal forms were also demonstrated which in unstained preparations resembled bundles of flagella interlaced, but they could be stained by ordinary methods, such as weak carbol fuchsine. Similar clumps of spirals had already been described by Loeffler (1889) and by Novy (1894). Circular, very thin-walled bodies were also seen ; these, too, were motile and suggested the appearance of plasmolysis, first described by Fischer (1900).

It is generally agreed that such forms as these are variants, and, so far, have not been found in the tissues of man, but they may be found in small numbers in most cultures—probably all—and it is possible that the thick ' bacteroid ' forms of Hort are more resistant than the small ordinary forms ; yet to build a complicated life-history on the foundation of the discovery of such forms, as has been done by Hort and other writers, is not yet justifiable on the evidence so far brought forward : yet, undoubtedly, this line of research holds out possibilities and should be pursued.

Mellon (1925) and Mellon and Jost (1926), who have published many papers on this subject, isolated a strain from the blood and fæces of a case of typhoid fever which showed small coccus forms on culture and was *non-agglutinable ;* but if grown on medium with $Na_2S_2O_3$, a luxuriant growth of long bacilli resulted. These were readily agglutinable by anti-typhosus serum. On replanting on agar without thiosulphate the coccal forms again appeared and were again non-agglutinable. Both Hort and Mellon, therefore, showed that with *B. typhosus* loss of motility, loss of flagella and change of morphology were associated with changes in agglutinability.

Gardner (1925) obtained branching forms of *B. typhosus* and other microbes by subculturing on agar from old stab cultures (10 months). He observed division of these forms under the microscope on agar blocks, and noted that separate individuals can be seen to be split off from each limb of the Y—' Three-point multiplication '. Gardner concludes : ' Up to the present I have come across no positive evidence that the Y forms are part of a complex life cycle. They appear, however, to be closely connected (in certain cases) with a variant or modification of the organism recognizable by the production of relatively small and opaque colonies, which consist largely of morphologically abnormal forms '.

Cultivation.

Temperature range. B. typhosus will grow at a wide range of temperatures : some growth is apparent at 4° C., but it is more copious, as already remarked, at from 35 to 40° C., with an optimum at 38° C. Growth ceases at 46° C., and all cultures are readily killed at slightly higher temperatures.

Reaction. Meyer (1921) found that *B. typhosus* grew in media with reactions over a range of pH 5 to 8·6 ; growth was most profuse at pH 6·8 to 7. The media used for production of vaccine in bulk is adjusted, as a rule, to pH 7·2.

Moisture. Moisture is necessary for growth, and, when it is absent or reduced, changes in morphology can be observed.

Oxygen. B. typhosus is a facultative anaerobe, but the growth in presence of oxygen is much more profuse than when the supply is small or non-existent. For example, in a stab culture into gelatin there is a profuse growth on the surface spreading out to the margin of the tube, and a scanty growth along the line of puncture, which becomes less and less the further the line extends from the surface ; growth practically ceases at the lower end. If the oxygen is carefully removed from a tube of broth by any of the ordinary methods for anaerobic culture and this tube is inoculated with *B. typhosus*, a faint growth appears after 48 hours, but it is never of the same order as in the open tube.

Gelatin. On gelatin *B. typhosus* grows readily, but does not produce so profuse a growth as on agar ; the growth may show a bluish tinge. There is no liquefaction of the gelatin.

Milk. *B. typhosus* grows readily in sterilized milk : the medium is not clotted, but if an indicator such as litmus is added it can be shown that a faint trace of acidity is produced in 24 hours ; as a general rule the milk becomes neutral or slightly alkaline in about 14 days.

Agar. On an agar slope of suitable reaction *B. typhosus* produces a luxuriant growth in 24 hours at 37° C., but not so luxuriant as that of *B. coli* or *B. paratyphosus* B., though rather more so than that of *B. paratyphosus* A. This growth is neither hard nor sticky, but small portions can be readily removed on the platinum needle without in any way drawing out the culture from the surface, as may be done with certain of the typhoid-like cultures of other intestinal bacteria.

On agar plates the colonies of *B. typhosus* after 24-hours' growth are small, clear and transparent, easily picked off and readily emulsified in saline. They are larger than those of streptococci, but distinctly smaller, clearer and more transparent than those of *B. coli* and *B. paratyphosus* B. ; they are larger and more veined than those of *B. paratyphosus* A.

It is not proposed to give here any elaborate description of the appearance of the growth of *B. typhosus* on agar, gelatin or other medium. In older days one had to rely on such descriptions or characters to distinguish between *B. coli* and *B. typhosus*, but now differential media, such as lactose-litmus agar, are employed and the two organisms can be readily differentiated.

It is interesting to note that many of the descriptions of colonies of the typhoid bacillus on agar plates which one finds in the older text-books and papers are descriptions of what are now called ' rough ' colonies. Take, for instance, the following description by Russell (1912) : ' Some clear colourless colonies on lactose-litmus agar, others, margin irregular and indented and surface veined ; ridges and valleys sloping away from the peak. Some, indeed, might be described as "barnacle" colonies '. He adds, ' the morphology of the colony varies according to the moisture, crowding, reaction and thickness of agar '. Another description of colonies is ' smooth, round, domed, shiny, translucent ', i.e. ' smooth ' colonies.

Broth. In broth the recently isolated culture of *B. typhosus* shows a diffuse turbidity in 24 hours, when the reaction and composition of the medium are optimum. There is, as a rule, no deposit and no scum, but in trypsin broth a rapidly growing culture is most dense at or near the surface where there is the maximum supply of oxygen. This may even suggest a scum, but on shaking the tube the bacilli can be easily disseminated through the medium, which shows some shot-silk opalescence if held up against a source of light.

A method of getting a maximum growth of *B. typhosus* in broth is by growing it for a few hours in a tube and then flowing the broth over the surface of a Roux bottle of trypsin agar and incubating on the flat. This gives the maximum growth in the thin layer of broth on the surface of the agar.

Potato. In older days a great deal of reliance in the identification of *B. typhosus* was placed on the growth on potato. This growth is colourless if the potato is good and the medium slightly acid, and is only apparent when the surface is stroked over by a platinum wire loop ; a small whitish ball of bacilli is rolled up in front of the loop, and if this is spread on a slide and stained the bacilli can be clearly seen.

Indole. There is no production of indole by any of the methods.

Special nutritive requirements. In vaccine preparation in bulk it is necessary to have as profuse a growth as possible. Whether such a growth is as good an antigen as one which is less luxuriant is a debateable question and one which might be investigated. If grown in ordinary beef infusion, the reaction of which had been adjusted by phenolphthalein and is, therefore, subject to variation, the growth varies from day to day and may never be profuse. This difficulty was overcome during the war by the use of Douglas' trypsinized ox-heart medium, which provides the optimum conditions for the growth of *B. typhosus*, when reaction is adjusted to pH 7·2.

A great variety of media have been experimented with by various authors, with a view to obtaining maximum growth, and various substances added to the media, such as trypsinized nutrose, but none has yielded such a satisfactory growth as Douglas' medium.

Colony variation—rough and smooth, &c. The general subject of bacterial variation is treated in Volume I, but so far as it affects *B. typhosus* it must be given some attention here, as a considerable amount of the work on ' variation ' or ' mutation ' has been done with special reference to the coli-typhoid group.

Hadley (1927) deals with this subject at considerable length under the title of ' Microbic Dissociation ', and reviews the literature. So long ago as 1890, Babes described the variability of typhoid colonies, and Baerthlein (1912) enumerated a great variety of types of colony for *B. typhosus*, one type shading off into the other. At the one end of the list was what is now known as the smooth colony and at the other the rough, various gradations filling in the gap. As already pointed out, many of the descriptions of colonies of *B. typhosus* in older text-books are descriptions of what are now known *in sensu* Arkwright as ' rough ' colonies.

The normal or smooth typhoid colony is the one which we are accustomed to see when a successful blood culture is plated out, or when subcultures are made therefrom on agar plates ; but if the same culture is grown in broth for 72 hours at 37° C. and then held at room temperature for some days, and plate cultures are made from time to time, certain colonies which merit the description ' rough ' will be found, and if these are carefully subcultured into broth they may retain their acquired characteristics. The rough colonies are described by Arkwright (1921) as showing a more or less jagged outline, they are flatter than the normal, and often have an irregular, rough or dull surface, and they are slightly opaque ; whereas the smooth colonies are round, domed and translucent.

The individual bacilli in these colonies cannot be differentiated by morphology. They are identical in sugar reactions and may be motile or non-motile. But the rough colonies deposit in broth and are spontaneously clumped in normal saline, whereas the smooth colonies give a turbid growth in broth and are readily emulsified in normal saline. They also differ in agglutinating and antigenic properties. The rough variants of the typhoid group are definitely less virulent than the smooth and they also differ in protective power when used to prepare a vaccine.

It is not clear whether cultures which have been obtained from bone abscesses of long standing, or from the centre of gall-stones, give rough colonies, but the experience of the Author was that the appearance of such primary cultures in no way differed from that of primary cultures from the blood of cases of fever ; this is a point which might be further investigated.

Another type of colony is that which shows daughter-colonies or small papillary outgrowths upon the older parent-colonies. These will be mentioned later when the fermentation reactions are discussed ; again, other colonies show clear spaces or lytic areas, and on these areas secondary colonies may arise.

Smell. It is usually stated that cultures of *B. typhosus* are odourless. This may be true of the growths in a test tube, but when large quantities are grown in Roux bottles, there is a very characteristic odour with which all who have worked at vaccine preparation are familiar.

Colour. The typhoid bacillus itself is without pigment or colour, but colonies and growth in ordinary agar may show occasionally a yellowish tinge.

Biochemical Reactions.

Action on Proteins.

Kendall and Bly (1922) point out that the *B. typhosus* in media free from carbohydrates which it can attack, produces ammonia and free alkali progressively from the protein in the medium. They add that the typhoid-dysentery group of bacilli appear to build their bodies from the ' polypeptic ' fraction of ordinary nutrient gelatin medium, rather than from the gelatin protein which is also present. If utilizable carbohydrate is present in the medium, the requisite energy of the microbes is obtained from it. It is generally considered that the *B. typhosus* does not split the protein in peptone or in egg albumen, but utilizes the nitrogen of the amino-acids in peptone.

Cornwall (1926) showed that if cultures of *B. typhosus* in nutrient broth are tested from time to time with lead acetate, it will be found that the amount of soluble protein varies with the age of the culture. Protein is used up by the bacilli, but more soluble protein is added in solution ; this must arise from lysis of the bacteria and the addition to the fluid of the protein of the bacillary bodies. At the end of 24 hours a large number of

bacilli have died and have been partially dissolved. The maximum concentration of bacilli is usually reached in 8 hours ; the numbers then fall. Very little nutriment is required to sustain life in bacilli, but a plentiful supply is required to produce reproduction. Living bacilli in old cultures, according to Cornwall, are probably only a few hours old.

Action on Carbohydrates.

The fermentation reactions in sugars of *B. typhosus* is, in normal conditions, very constant. For instance, the reactions of *B. typhosus* (Rawlins) isolated from the blood of a case of fever nearly 30 years ago and subcultured at weekly and, during the war, daily intervals are precisely the same as those of recently isolated cultures, i.e. acid without gas in glucose, mannitol, maltose, dextrin, galactose, sorbitol, lævulose and xylose. No change in lactose, dulcitol, saccharose, inulin, raffinose, arabinose, salicin and erythritol. Milk is acidified but not clotted.

Twort (1907) has shown that the sugar-fermenting powers of an organism may be artificially produced or altered by growing it for a succession of generations in media containing a sugar which at the commencement of the experiment it was unable to ferment. After two years' ' training ' on lactose, subculturing once a fortnight, *B. typhosus* acquired the power of fermenting this sugar, with production of acid ; but again lost the property on return to non-lactose medium. The same applies to dulcitol and arabinose, but the power is much more rapidly acquired.

Penfold (1910) showed that *B. typhosus* is a dulcitol fermenter. It ferments dulcitol-peptone after ten days, but if subcultured on dulcitol medium the period is shortened to one day. It produces red papillæ on dulcitol-McConkey media ; if subcultures are made from the papillæ they ferment dulcitol. The same applies to those strains which do not ferment glycerol on first trial.

Mandelbaum (1912) isolated a culture of *B. typhosus* from the blood, which produced alkali in glycerol but was otherwise typical.

Teague and Morishima (1920) and Morishima (1921) tested a very large number of strains of *B. typhosus*. They found that all could ferment xylose, although about 8 per cent. were slow fermenters ; but if the latter were subcultured for a few generations on xylose medium they readily fermented this sugar. On arabinose, 94 per cent. of the strains did not ferment the sugar, 6 per cent. did ; but on solid media they produced daughter-colonies, showing that they could utilize the sugar. Morishima considers that the production of daughter-colonies implies that the sugar has been utilized, e.g. rhamnose agar, dulcitol agar.

Sears and Putnam (1923) showed that *B. typhosus* growing in symbiosis with *B. proteus vulgaris* produces gas in mannitol. Neither of these bacilli can do this alone, nor does it occur if first one bacillus is grown in the medium and later on the other is grown in the same medium after filtration. Castellani (1927) stated that with *B. typhosus* in symbiosis

with *B. morgani* in a maltose tube gas may be produced, although neither produces gas in maltose when grown in pure culture. He suggests that this may be due to the fact that *B. typhosus* produces formic acid from maltose and this can be fermented with production of gas by *B. morgani*. Already in 1911 Penfold had shown that when his *B. coli* variant (obtained by growth on monochloracetic acid agar) which was unable to produce gas from sugars was incubated in symbiosis with *B. typhosus* in a glucose medium, gas was produced, and in the light of Harden's work, he concluded that the formic acid known to be produced by *B. typhosus* from glucose was split up by the variant *B. coli*. The selective process on the monochloracetate agar had removed the formic-acid-forming ferments, but apparently had not interfered with the formic-acid-splitting ferment. The symbiosis experiments of Castellani depend doubtless on similar ferment mechanisms.

Serological Reactions.

Agglutination.

The technique of this reaction is described elsewhere. Some workers prefer to use a living broth culture or a living saline suspension from a young agar culture. Dead bacteria are, however, as readily agglutinated as living ones and in some respects are more convenient, as emulsions can be kept in stock and are safe to handle. It should always be remembered that if a change over from one emulsion to another is necessary, it is essential to compare the agglutinability of the new batch with the old.

Birt and Lamb (1899), working at Netley with Wright, were probably the first to insist on the necessity of standardizing agglutinability of emulsions. They pointed out that if comparable results are required it is necessary to use the same (killed) emulsion for each estimation. They ensured this by making up sufficient killed emulsion to last for some time. They also pointed out that if for any reason it was not possible to use the same emulsion throughout, it would be necessary, before taking a new batch into use, to compare the agglutinability of the new with the old, and when carrying out further tests to make allowance for any difference found.

Absorption of Agglutinins.

B. typhosus, as stated above, is, as a rule, so clear-cut in its reactions, both serological and biochemical, that resort to this further test is not necessary; but if there is any doubt as to the identity of a bacillus isolated from the fæces it should not be accepted as true *B. typhosus* until it has been shown to be capable of removing the specific agglutinins from a high titre *B. typhosus* serum, and also of producing readily such agglutinins when inoculated into rabbits.

Another use of this test is to remove secondary agglutinins from high titre sera by absorption with the bacillus concerned, thus leaving a pure *B. typhosus* serum.

Variations in Agglutinability—Inagglutinable Strains, &c.—Loss of Agglutinability.

It is well known that some strains of *B. typhosus* when first isolated from the blood of a case of fever may be found to be inagglutinable, or feebly agglutinable, but they may regain or acquire agglutinability after subculture. Also agglutinable strains if grown in or on immune serum may become inagglutinable.

McIntosh and McQueen (1914) isolated from the blood of a case of typhoid fever a strain of *B. typhosus* which was completely inagglutinable by the serum of the patient and also by high titre specific serum ; otherwise it presented all the characteristics of *B. typhosus*. The serum of the patient agglutinated a stock strain of *B. typhosus* 1/50 in five minutes. A rabbit was inoculated with an emulsion of the inagglutinable strains and agglutinins were produced for the stock *B. typhosus*, but the serum of the rabbit failed to agglutinate the strain with which it had been inoculated. Its agglutinogenic functions were, therefore, unimpaired. Some group agglutinins were also produced in the serum for Gaertner's bacillus. This inagglutinable bacillus was capable of absorbing all the specific and group agglutinins from the homologous serum. This absorbed agglutinin could again be removed from the bacillus by digestion with serum. There was no difference between the normal and inagglutinable strains with regard to acid agglutinations, showing that some of the physical properties of the bacillus were interfered with whereas others were not.

Blankenhorn and others (1923) isolated a slowly agglutinable strain of *B. typhosus* from a periosteal node on the tibia during the course of an atypical fever. Cultures from the blood, fæces and urine were negative. The Widal test was negative.

The bacillus was not agglutinated by its own serum or by stock typhoid serum, but it removed agglutinins for stock strains from specific serum. If the tubes were left over night it agglutinated up to 1/2,000.

Floccular and Granular, Somatic and Flagellar Agglutination.

Malvoz (1897) associated loss of agglutinability with loss of motility and attributed the loss to the fact that the methods he employed deprived the bacilli of flagella.

Ainley Walker (1918) showed that certain strains of *B. typhosus* differed greatly in agglutinability, and Dreyer (quoted by Gardner) has observed that it is possible to select from a single culture, individuals which will produce populations differing as widely serologically as any two strains obtained from different sources.

Feiler (1920) grew *B. typhosus* on carbolized media and found it to be without flagella, and no S or H floccular agglutinins or agglutinogens could be demonstrated ; the serum of animals inoculated with emulsions from this culture showed only granular agglutinins. He gave the names ectoplasmic agglutinins and endoplasmic agglutinins to these. The carbolized strain absorbed only part of the agglutinins from specific serum prepared

from normal bacilli. Gardner and Ainley Walker (1921) verified Feiler's observations. They selected four strains of *B. typhosus*—T.T., T.O., T.L. and T.E.—and prepared agglutinable cultures therefrom and standardized these. A rabbit was given three doses of 25,000 bacilli—T.L. on the first day, T.E. on the eighth day and T.O. on the sixteenth day. The rabbit was bled and the serum tested against all four cultures. They fell readily into two groups—T.O. and T.T., T.L. and T.E.—and the characters which distinguished the strains were non-motility and comparative inagglutinability. The motile forms, T.O. and T.T., agglutinated in large flocculent clumps : the non-motile T.E. and T.L. in small, compact, granular clumps. They also observed that these characters are only temporary, as a non-motile form of *B. typhosus* will eventually produce a motile, and vice versa, and each individual bacillus, whether motile or non-motile, possesses both potentialities. The practical point that emerges is the necessity for the careful selection of cultures for agglutination tests, and their ' fixation ' by formalin, and standardization by sera of known titre.

Burnet (1924) concludes that the living typhoid bacillus contains two antigenic constituents concerned in agglutination reactions. S or H agglutinogen, probably represents in part the flagellar protein, and is thus readily removed from the bacillus by various physical and chemical means, and does not appear at all under certain conditions of growth. R or O agglutinogen is probably connected with the specific body protein of the bacillus and is much less susceptible to physical and chemical agents. Standard emulsions used for agglutination contain bacilli which possess both antigens as part of their specific structure. The bulk of the S or H agglutinogen is in solution and part of the R or O agglutination of this emulsion by serum containing S agglutinin is largely a precipitation reaction, and the aggregation of the bacilli is chiefly due to their entanglement in the meshes of the light coagulum so formed.

Felix (1924) used three cultures—T9 01, T1 and G2 (Gaertner). T9 01 was a specially selected culture of *B. typhosus* which was sensitive to granular agglutination alone ; T1, the ordinary type, showed both floccular and granular agglutination ; G2 (Gaertner) recorded only granular typhoid agglutination. Felix found that small granular agglutinins appeared in the serum earlier in the disease than the floccular ; thus the use of T9 01 gave an earlier diagnosis. The entire absence of small granular agglutinins from the serum of a case of fever excludes a diagnosis of typhoid fever in Felix's opinion. Thus, as granular agglutination is common to various bacteria, e.g. Gaertner, *paratyphosus* A, *paratyphosus* B and *B. typhosus*, a differential diagnosis by Felix's method is not possible in inoculated persons : one can only say ' enteric group ? '. This in his opinion applies also to non-inoculated cases of fever which only show small granular agglutination and no floccular ; and T.A.B. inoculations, in man, lead only to the production of large floccular agglutinations in the serum of inoculated persons. Felix also considers that inagglutinable or slowly agglutinable strains are produced by the loss of the large floccular antigens ;

if small clumping agglutinins are present in the blood, successful blood culture is much more difficult to obtain, as the number of bacilli is much less than in cases which show much flocculent agglutination. Agglutination can be used as a prognostic : the appearance of small flaked agglutinins before the end of the first week of the fever is of favourable significance. Felix associates the granular agglutinins with immune body, and as in his experience typhoid vaccine does not produce these in man he considers that such vaccine cannot and does not protect ; and also he and others with him do not believe that successful blood culture is more difficult to obtain in the inoculated than in uninoculated.

Orcutt (1924) has shown that the H type of agglutination is almost invariably associated with the motile phase and is clearly associated with the flagella ; by shaking and subsequent centrifugalizing, the bodies of the bacilli are thrown down and the flagella are left in the glass-clear supernatant fluid. Injection of this fluid or the whole bacilli produces a flocculating serum ; the injection of the bodies alone produces a granular agglutinating serum. When the supernatant fluid is heated to 70° C. or over, it can be demonstrated that the flagella are destroyed and the fluid no longer flocculates, but it can still produce agglutinins in the blood of animals into which it is inoculated. Bruce White (1926) is likewise of opinion that loss of flocculating power is associated with loss of motility, i.e. loss of flagella.

Arkwright (1921), in his work on rough and smooth colony variations, showed that this change involves differences in serological reactions. But the change from smooth to rough causes, as a rule, no change in the flocculating antigen. Bruce White considers that the change from smooth to rough involves reduction (quantitative and sometimes qualitative), or even total loss of, the normal O stable or granulating antigens and the appearance of new O and equally stable antigens peculiar to the rough state. This antigenic modification is reflected in the nature of the corresponding sera. But there is no alteration of the flocculating antigen.

In this connection Schütze (1921) points out that ' there exists a serological cosmopolitanism among rough cultures '. For example, rough variants of the bacillus of Gaertner, *B. paratyphosus* A and typhoid strains will agglutinate sometimes to titre limit with rough sera of the paratyphoid group, whereas the smooth strains show no serological relation whatever, i.e. the floccular agglutinin is specific : but by absorption even the more closely related of the heterologous rough strains can be differentiated and the homologous ones identified. The method employed by Schütze is to prepare a serum from the unknown rough strain, and then absorb this with various known rough strains. The one which removes the agglutinins gives the clue to the identity of the unknown culture.

Arkwright (1927), in his most recent work, discusses the whole question of variation. This variation may affect morphology of the individuals and of the colonies, the manner of growth in solid and fluid medium, biochemical reactions, toxin production, serological reaction and virulence.

But he adds that some bacteria are remarkably constant as regards their antigenic constituents and the corresponding antibodies, e.g. *B. typhosus*. He divides the serological properties of *B. typhosus* into three classes of antigen.

1. H., or heat-labile at 100° C.= flagellar.
2. S.O., or heat-stable at 100° C.= somatic.
3. R.O. form, heat-stable at 100° C.

S heat-stable and R heat-stable antigens are serologically distinct. The H, or flagellar antigen, is the same in rough and smooth cultures when they are motile.

There are thus four types :

S, motile = S.M.
S, non-motile = S.N.M.
R, motile = R.M.
R, non-motile = R.N.M.

He found that the smooth motile and smooth non-motile cultures were more virulent than the rough cultures, whether motile or not, and that the H heat-labile antigen and R heat-stable antigen are of little or no value in producing immunity in experimental animals as tested by intraperitoneal inoculation.

Serological Relationship with Certain Members of the Salmonella Group. Cross Agglutination.

Smith and TenBroeck (1915) showed that *B. typhosus* and a strain of fowl typhoid were closely related serologically, although there were certain cultural differences, and the fowl typhoid strain was non-motile. This work, it should be noted, was carried out before the difference between, and the significance of, rough and smooth variants had been fully appreciated, and it is not certain what type of *B. typhosus* was utilized, but it is stated that the culture was typical in regard to fermentation reactions, motility, &c. The fowl typhoid strain was probably *B. gallinarum* (*sanguinarium*).

The authors prepared sera by inoculation of cultures into rabbits, and found that there was marked cross agglutination : the fowl typhoid serum agglutinated *B. typhosus* to practically the same titre as it did the homologous organism, and vice versa. They also noted that the serum produced by the motile organism (*B. typhosus*) clumped the motile organism in loose and flocculent clumps, but agglutinated the non-motile bacillus in compact granular clumps ; whereas the serum produced by the inoculation of the non-motile fowl typhoid clumped both motile *B. typhosus* and the non-motile *B. sanguinarium* in compact clumps. Although this does occur to a certain extent, the phenomenon is not so clear-cut as the authors state ; but at any rate the close serological interrelationship of these two bacilli, *B. typhosus* of human origin and *B. sanguinarium* of fowl typhoid, was clearly demonstrated.

Absorption experiments. It was found that *B. typhosus* removed the agglutinins from *typhosus* serum and from fowl typhoid serum, both for itself and for fowl typhoid. But the fowl typhoid did not reciprocate, removing only agglutinins for itself, not for *typhosus*, i.e. the non-motile bacillus was unable to remove flagellar agglutinins. They also found that serum from typhoid patients may agglutinate fowl typhoid, but not to so high a titre as it does *B. typhosus*.

Bruce White (1926) has discussed the relationship of the Salmonella group and allied organisms. He points out that *B. typhosus* is one of the monophasic organisms in the sense of Andrewes, thus differing from the majority of the Salmonellas, which are diphasic. *B. paratyphosus* A, *B. enteritidis*, and the fowl typhoid bacilli are monophasic, and they are most closely related to *B. typhosus* serologically and in other respects. If *typhosus* had been diphasic one might have looked here for a possible explanation of the relapse in typhoid fever, namely, that the original attack is due to the specific phase : and that as soon as the antibodies to this phase are developed, the relapse is produced by ' switching over ' to the non-specific. But there is at present no evidence of this, and it is a subject for further investigation. White noted that there is evidence from the history of food-poisoning outbreaks that typhoid convalescents are to some extent more resistant than normal people to Gaertner infections. It has also been shown that the serum of patients recently recovered from typhoid fever protects guinea-pigs from doses of *B. enteritidis* which are fatal to controls ; and rabbits protected against *B. typhosus* are resistant to large doses of the ' Stanley ' strain, the latter being a diphasic member of the Salmonella group.

These cross-immunization experiments show a close relationship between *B. typhosus* and those two species of bacteria, which are sharply differentiated from it by the fermentation reactions. *B. typhosus* and ' Stanley ' also show a close serological relationship. Thus a serum produced by inoculation of *B. typhosus* into rabbits agglutinated an emulsion of the specific phase of ' Stanley ' to a titre of 1/10,000, the agglutination being of the flocculent type. Absorption of this serum with specific ' Stanley ' strain removed all or practically all the floccular agglutinins for *B. typhosus*.

The serological relationship of *B. typhosus* with Gaertner's bacillus is also close, but of a different character. Here it is the granular or heat-stable agglutinins which can be correlated, and the same phenomena of cross-absorption and agglutination can be demonstrated as with the ' Stanley ' strain. For this purpose, cultures grown on phenol agar and subsequently heated to 100° C. are utilized. The bacilli do not develop flagella on this medium, thus they are free from the floccular agglutinin-producing factor, but retain the granular heat-stable agglutinogens and agglutinins.

Simple agglutination tests made with *B. sanguinarium*, *B. pullorum* and *B. enteritidis* sera showed that none of these differentiated in their

actions between *B. pullorum*, *B. sanguinarium* and phenol-agar cultures of *B. enteritidis* and *B. typhosus*. Sera prepared from phenol-agar cultures of *B. typhosus* acted in the same way, but those from ordinary unheated cultures of *B. typhosus* acted far more vigorously, i.e. to higher titre, on the homologous *B. typhosus* emulsion than on the heterologous. A serum for *B. gallinarum* was almost completely divested of agglutinins by absorption with *B. pullorum*, *B. enteritidis* or *B. typhosus*, and *B. enteritidis* sera were robbed of their O (granular) agglutinins by treatment with *B. gallinarum* or *B. typhosus*. But White found that the O agglutinins in *B. typhosus* serum were not entirely removed by absorption with *B. enteritidis* or with *B. gallinarum* or *B. pullorum*.

Thus we have here the double relationship of *B. typhosus* with the Salmonella group : on the one hand with the specific phase of the strain ' Stanley ' by means of floccular agglutination ; on the other hand with *B. enteritidis*, *B. pullorum* and *B. gallinarum* or *B. sanguinarium* by means of granular agglutination and absorption of the same.

Fortunately, the strain ' Stanley ' is of very great rarity and can be readily distinguished by the sugar reactions.

Complement Fixation.

Bordet, in his original experiments on this reaction, used a saline emulsion of *B. typhosus* as antigen. In more recent work, Matsumoto (1920) tested a variety of antigens and arrived at the conclusion that, in his hands, the best was a 14-day old broth culture of *B. typhosus* heated to 60° C. for one hour with 0·5 per cent. phenol added. The next best was an emulsion of living typhoid bacilli in 0·8 per cent. saline.

Felix (1924) is of opinion that the complement-fixation reaction gives a more accurate indication of the immunity curve than does the agglutination test, and that it follows the curve of the granular agglutination rather than the floccular, and does not appear in the blood of inoculated persons ; but this is contrary to the experience of other workers. Menton (1926) refers to a case in which the complement-fixation test was positive five years after inoculation.

Hooker (1916) investigated a number of strains of *B. typhosus* by the complement-fixation method, and found that the older strains were less efficient as antigen than those more recently isolated. They fell into three groups, and the absorption test confirmed this grouping. Sera immune to any recently isolated strain cross fix with all other strains, old or young, while sera immune to older strains do not so cross fix. The logical conclusion is that a young strain ought to afford the most efficient protection when used for prophylactic immunization against typhoid fever.

Bull (1916), for complement-fixation tests, used an antigen prepared as follows : *B. typhosus* was grown on agar and washed off in saline ; the washed bacillary bodies were frozen and thawed several times, dried in vacuum over sulphuric acid and ground to a fine powder in an agate

mortar ; 1 dgm. of the powder was added to 100 c.cm. of salt solution, then shaken for several hours and filtered. The clear filtrate was used both for complement-fixation tests and for precipitin tests. It proved highly satisfactory.

Precipitin Reaction.

This reaction has also been employed in the diagnosis of typhoid fever. If the clear filtered broth from a culture of *B. typhosus* is added to the serum of a convalescent from typhoid fever, or to the serum of an animal which has been inoculated with *B. typhosus*, a fine cloud appears in the tube, and if left to stand for some time a deposit forms at the bottom of the tube.

Bactericidal Substances.

Bactericidal and bacteriolytic substances for *B. typhosus* can be readily demonstrated in the sera of patients or in the blood of animals or men inoculated with the bacillary vaccines. The technique of these tests is described elsewhere.

Normal Antibodies in Man and Animals.

Before taking any animal into use for experimental purposes it is essential that careful tests should be made for agglutinative or bactericidal powers of the blood, as these may be present in the sera of normal animals. With modern methods of testing the agglutination titre of the serum, it may be taken that if agglutinins are present in human blood they are the result either of an existing or previous infection, or possibly of an inoculation some years earlier.

Pathogenic Action on Experimental Animals and Man.

Animals.

So far as is known no animal other than man in Nature suffers from true typhoid fever. But inoculation of *B. typhosus*, although it does not produce typhoid fever, can, and does, produce a septicæmia or toxæmia in rabbits, mice and other laboratory animals, and can kill them. Chantemesse and Widal (1892), who carried out some of the earliest experiments on animals, noted that if a sublethal dose was given, the animals which recovered showed an increased resistance to fresh inoculations with the bacillus as compared with control animals. Gilbert and Girode (1891), about the same time, describe in guinea-pigs, inoculated intraperitoneally with *B. typhosus*, post-mortem appearances suggestive of typhoid fever, i.e. ulceration of lymph follicles and swelling of the mucous membrane of the small intestine.

Sanarelli (1892, 1894) found that in order to kill rabbits it was necessary to raise the virulence of the bacillus by passage. This he did by giving a massive dose under the skin, sufficient to kill. He then recovered the bacillus from the peritoneal fluid, cultured it and again inoculated under

the skin. By this means the dose could be reduced so that 0·75 c.cm. would kill a rabbit. He also showed that if a sublethal dose of *B. typhosus* was inoculated subcutaneously and a filtered culture of streptococci or *B. coli* inoculated intraperitoneally, death resulted, and in the killed animals Peyer's patches were enlarged. There was also an acute desquamative enteritis and *B. typhosus* was found in the blood, in the fæces and in the peritoneal exudate. If a sublethal dose of *B. typhosus* was inoculated subcutaneously an abscess would result; later, when this abscess had apparently healed, the injection of *B. coli* toxin intraperitoneally might cause a generalized infection.

Metchnikoff and Besredka (1911) showed that chimpanzees could be infected by the mouth by massive doses and that fever followed and in fatal cases lesions resembling those in man resulted. But, as Besredka himself says, the higher ape is ' animal de luxe ' and not available in large numbers.

Much of the more recent work on typhoid infection in animals has been carried out on rabbits and with special reference to the production of the carrier state. That rabbits could become carriers of *B. typhosus* has been known for many years (Blachstein, 1891) and, indeed, was well known before the significance of the carrier state in man was fully appreciated. Morgan (1911) inoculated rabbits intravenously and recovered the bacillus from the bile, but was only able on two occasions to recover it from the fæces. Difficulty in regularly recovering the bacillus from the fæces has been experienced by other workers, and, for this reason, the rabbit has not proved valuable for the testing of cures of the typhoid carrier condition.

Cummins and Cumming (1914) selected 10 rabbits ; 5 were immunized by the injection of 50 million killed *B. typhosus* on two occasions at 10 days interval ; agglutinins and thermo-stable opsonins were noted in the blood. Five were kept as controls. A fortnight after the second dose of the vaccine all 10 rabbits were given a test dose of 1/10 of an agar slope of living *B. typhosus* intravenously. Two rabbits, one immunized and one control, were killed at intervals of one week and examined. Of the immunized animals in two instances the bacillus was recovered from the liver and in one there was necrosis of the wall of the gall-bladder. The results in the controls were practically the same.

Meyer (1921) and others have published a series of papers dealing very fully with this subject and reviewing the literature. They found that there was no striking difference in their potentialities as carrier producers in animals of strains of *B. typhosus* isolated from case or from carrier in man. Strains isolated from human gall-bladder carriers showed no elective tendency to infect the gall-bladder of the rabbit, nor did the bacilli isolated from the urine of a human carrier show any particular affinity for the kidney of the rabbit. A small number of typhoid bacilli inoculated intravenously into the rabbit disappeared from the blood in 10 to 15 minutes. The animals were then killed and the number of bacilli present in various

tissues and organs of the body was estimated by taking small portions of such organs, weighing and then grinding up and plating measured amounts. By this means it was found that from 20 to 30 per cent. of the inoculated bacilli could be recovered from the liver, the majority of the remainder from the spleen, bone marrow and lungs, and a few from the kidneys, lymph nodes and muscles. At this early stage of infection only a few could be found in the bile, but they were numerous in the wall of the gall-bladder.

It was further found, by killing animals at various periods after inoculation, that the bacilli were rapidly destroyed in the liver, spleen and lung, but not so readily in the bone marrow, where they may multiply and form foci which reinfect the blood-stream at a later date. As regards the gall-bladder, the bile may be infected in three ways : (1) directly, by eliminated bacilli ; (2) from foci in the liver ; (3) from necrosed areas in gall-bladder wall.

Other interesting points noted by Meyer and his colleagues were that rabbits which succumb to typhoid intoxication, regularly harbour large numbers of bacilli in the bone marrow of the long bones ; also, the bile may be sterile, although the walls of the gall-bladder are teeming with bacilli. Calculi occurred in 60 to 80 per cent. of their infected rabbits. These calculi consisted of bilirubin and calcareous material. Thirty to 40 per cent. of intravenously inoculated animals recovered in a month ; 10 to 15 per cent. retained bacilli in the bile for six months to a year or longer. In rabbits the persistence of the carrier state depends on the degree of inflammation of the gall-bladder wall.

In a further paper of the series, Meyer discusses the question whether there is any cultural or serological difference between carrier strains and strains isolated from the blood of acute cases. Metchnikoff and Besredka are stated to have found that they could not infect chimpanzees by the mouth with carrier strains, although these carrier strains killed small laboratory animals when inoculated in sufficient dose. Other workers hold that carrier strains are not so toxic as strains isolated from cases of enteric fever. Meyer could not make out any difference either culturally or biochemically. He was unable to ' educate ' a carrier strain by a stay in the gall-bladder of a rabbit. But he arrived at the conclusion that strains recently isolated, whether from the excreta of a carrier or from the blood of a case of fever, produced a higher percentage of carriers when inoculated intravenously into rabbits. He also found that bacilli were killed or disappeared from the blood of normal animals just as readily as from the blood of immunized animals. A dose of over 1,000 million bacilli was necessary to ensure discharge of the bacilli in the bile. There was also some evidence, both from Meyer's own work and from the literature, that there was a higher proportion of carriers among immunized animals than in the normal.

Meyer found that subcutaneous injection of a large dose of bacilli failed to cause a general infection or to produce carriers. The disease produced in rabbits by an intravenous injection of bacilli is a toxæmia rather than

a septicæmia. The toxins are of cellular origin, being the product of interaction between the cells of the tissues and the disintegrated bacilli. The greater the destruction of the bacilli the more is the animal intoxicated; but immunized animals can resist this toxæmia and thus prevent invasion of the bone marrow.

Meyer sums up by saying that in the rabbit the real chronic carriers are, as a rule, those in which the gall-bladder wall is infected and there are gall-stones present, and not merely the bile-infected animals. On histological examination the mucosa of the gall-bladder is found to be thickened and the papillæ are hypertrophied; there may even be an empyema of the gall-bladder. In some cases intrahepatic foci may keep up the infection of the bile even if the cystic duct is blocked.

Some of their rabbits remained carriers for from 100 to 800 days.

The hæmato-hepatogenous route of infection is the usual one in the rabbit and probably also in man. Cornwall and LaFrenais (1924) injected living typhoid bacilli directly into the jejunum of rabbits and were able to recover them from the general circulation within an hour, and hourly up to 30 hours. The bacilli then disappeared from the blood and took up their abode in the liver and spleen, where they are rapidly destroyed. Rabbits may free themselves completely in seven days after a jejunal injection of *B. typhosus*. Only small numbers of bacilli pass through the jejunal wall into the general circulation. They can survive in the small intestine and multiply for one month, and can pass quickly through the intestinal mucosa into the general circulation. Intravenous injection caused rabbits to become carriers, the bile being infected in the liver and carried to the gall-bladder and thence to the intestine. Cornwall also found that the destruction of many bacilli leads to the production of much toxin, with sometimes fatal effects. The intravenous injection of dead bacilli was just as fatal to rabbits as a similar dose of living ones. In Cornwall's view the immunity of the animal depends on the ability of the intestinal mucous membranes to resist the passage of *B. typhosus* and the ability of the tissue cells to withstand the noxious effect of toxins liberated by the death of the bacilli.

Besredka (1919) points out that rats, mice, guinea-pigs and rabbits are all susceptible to subcutaneous, intravenous and intraperitoneal inoculation of *B. typhosus*, but not to infection by the mouth. Even massive doses by this route have no effect, except a slight loss of weight. This is probably due to the acidity of the stomach contents, as shown later by Cornwall and others. Besredka considers that the wall of the small intestine acts as a barrier to the passage of bacilli and that administration of bile so alters the mucosa as to permit the passage of bacilli. Bile sensitizes the rabbit to the bacillus by causing an intense desquamation of the mucous membrane of the small intestine, yet bile alone is innocuous. With small doses of virus *per os* after administration of bile, a fatal infection is set up following an incubation period of 4 days, and the animal dies after 6 to 16 days. *Post mortem* the small intestine is seen to be transparent and

congested ; the stomach contents are sterile, but numerous bacilli are found in the bile and in the small intestine. In the large intestine they cannot be found.

Veratti and Cattaneo (1920), on the other hand, showed that bile does not desquamate the intestine, but does sensitize animals—that is, they are more readily infected by smaller doses of virus after a preliminary dose of bile. Cornwall considers that this may be due to interference with the acid barrier of the stomach, as he showed that the bacilli pass readily through the mucous membrane of the small intestine when directly injected therein.

Ledingham (1926) gives details of some previously unpublished work on the carrier state in animals. He utilized guinea-pigs and injected a dose of *B. typhosus* directly into the gall-bladder, and he also succeeded in infecting the bile by the intraperitoneal route. In one instance he injected a guinea-pig intravenously, and, although this particular animal did not become a gall-bladder carrier, Ledingham was able to recover *B. typhosus* from a periosteal node on a rib. Four guinea-pigs were inoculated directly into the gall-bladder. One died on the thirty-fifth day and the bile and heart-blood yielded positive cultures ; one was killed on the sixty-third day but the bile culture was negative; however, the bacillus had been successfully isolated from the fæces on the fifteenth, nineteenth and twenty-second days.

Virulence and Toxicity.

Among others who worked on the toxins of *B. typhosus* were Brieger (1902), Luff (1889), Sanarelli (1894), Chantemesse (1897), Pfeiffer (1894), and MacFadyean and Rowland (1903). The last-named authors froze emulsions of the bacillus at the temperature of liquid air, ground them up, and obtained from them what they considered to be a true endotoxin ; incidentally, also, they found that all bacilli were not killed at this temperature. In more recent times a considerable amount of work has been done on the subject, and especially in America, where the theory of Vaughan and his co-workers on anaphylatoxins has received support.

Jobling and Bull (1913) discuss the true soluble toxins of diphtheria, tetanus and *B. welchii*. They show that these are specific secretory toxins, and in the diseases caused by these bacteria we have local bacteria with a general toxæmia. *B. typhosus* does not secrete a true soluble toxin ; its toxic effects depend on a mechanical or chemical breaking down of the bodies of the bacteria ; nor so far has an antitoxin been produced to its toxins.

Nature and Source of Toxic Substances.

Pfeiffer (1894) first advanced the opinion that typhoid toxins are produced by destruction of the bacterial bodies by lysis in the body of infected animals, and that a thermolabile endotoxin is produced. But he he could find no toxin in filtrates of young broth cultures. Buchner also

prepared toxic substances from filtered extracts. Vaughan (1906) produced a toxic substance, soluble in alcohol, from typhoid bacilli, and from other bacteria some of which were non-pathogenic, and also from non-bacterial proteins. He considered these substances to be toxic albumoses. A dose of 8 mgm. killed guinea-pigs if given intraperitoneally. All these toxic products produced practically the same symptoms and were thus non-specific. Vaughan's idea was, not that the toxic substance was stored up in the interior of the bacterial bodies, but that it was a product of the splitting of the bacterial protein by the action of ferments of the infected animal, i.e. the typhoid bacillus neither contained nor secreted a specific toxin.

Jobling and Bull (1913) set out to discover whether these toxic substances were preformed in the bacteria or whether they were formed by a cleavage of the protein constituents of the bacterial bodies. In experiments on dogs they found that the same symptoms were caused by intravenous injection of either killed (boiled) culture, living bacilli, or bacilli completely dissolved by alkali. They judged, therefore, that the toxic substance was bound up in the bacteria. Filtered washings of young cultures were found to be non-toxic, but emulsions treated with leucocytic ferment, even if filtered, were highly toxic to dogs in amounts of 1 c.cm. to 2 c.cm. The animals died in from 2 to 6 hours. These toxic substances were thermostable.

Two possibilities were suggested and considered—(1) that the toxic substance is liberated by destruction of the bacterial bodies, and (2) that the toxic substance is produced by the action of the ferment on the protein of the bacteria. The latter supposition was the more favoured. They found that the ferment alone was not toxic. But the toxic substance produced by the action of the ferment on the bacteria meantime gradually became more toxic until the fifth day, and thereafter gradually diminished in toxicity. After 20 days, it was only slightly toxic. It is probable that the same is true in the body of the animal ; the leucocytes secrete a ferment which splits the protein of the bacteria and forms toxic substances, giving rise to fever, &c. Although the effects of this toxic material are apparently specific, in that they seem to have a special effect on the mucous membrane of the small intestine and especially on the lymph tissue, yet practically identical results were obtained by Bull when using toxin prepared in a similar manner from *B. coli* and *S. aureus*. That is, the pathological picture was a non-specific one.

Zinsser (1913) also refers to the endotoxin theory of Pfeiffer, but doubts if it is necessary to explain the toxic symptoms. He also showed that dead bacteria and bacterial extracts of *B. typhosus* had a strong toxic action, this action being due to poisonous ingredients in the cell bodies. These only appear after proved cleavage of the bacterial bodies brought about by the action of serum components. But he considers that true endotoxins may exist in addition to the above, thus giving a further specific element to the clinical picture of the disease.

Zinsser also found that prolonged exposure of sensitized and unsensitized bacilli to complement (15 hours) gives a powerful toxin, and this is not changed into non-toxic substance as is the general rule ; it was fairly stable. The name ' anaphylatoxin ' has been applied to this toxin. Zinsser compared it with the toxic substances obtained from typhoid bacilli by other methods. Filtrates of 10-day-old cultures in alkaline broth were found to be highly toxic for guinea-pigs, death occurring in 72 hours after an intravenous dose of 5 c.cm. of the filtrate. But if the culture was grown for two months and then filtered the filtrate was only slightly toxic. The washings from mechanically shaken bacilli were practically non-toxic ; but when the centrifuged bacilli were ground up in a freezing mixture and then suspended in 0·9 per cent. saline, the milky fluid was highly toxic for guinea-pigs, a dose of 2 c.cm. killing in 6 hours. The symptoms following the administration of these autolysates differed from the symptoms caused by anaphylatoxin obtained by the action of complement on sensitized bacilli, but in Zinsser's view this difference is rather one of degree than of kind.

Rosenow (1912) considers that the toxic substance of *B. typhosus* is probably a split product of the bacterial protoplasm. Ether helps to kill the bacteria and aids the autolytic changes. An emulsion of *B. typhosus* in normal saline under ether is specially toxic in 2 hours, but after 48 hours is only slightly toxic. This toxic substance can be produced by the action of immune and normal serum, and complement is not absolutely essential. Rosenow believes that in infections small numbers of bacteria are being continually broken down and thus give rise to a succession of small doses of toxin, so symptoms of fever, &c., are produced and not profound shock as in anaphylaxis. Other solvents, such as lipoids, may serve to extract the toxic substance from bacteria. In intravascular (intravenous) injections the toxic products are carried direct to the cells, whereas in subcutaneous injections the bacterial proteins are converted into non-toxic substances before they can be absorbed. The same applies to intravascular infections and local infections.

During the growth of typhoid bacilli the albuminous fluids in which they grow are converted into toxic cleavage products. *B. typhosus* was cultivated in alkaline broth for 10 days and then filtered. Five c.cm. of this filtrate injected into a rabbit of 1,350 gm. produced diarrhœa (fæces streaked with blood), weakness, paralysis of hind legs and death during the night after inoculation. This syndrome is not comparable (nor were other illnesses due to extracts, saline, &c.) to the effects of anaphylatoxin, but was more like typhoid fever, as hæmorrhages, &c., were noted.

Douglas (1921) carried out some experiments on the cleavage products of *B. typhosus*, and found that an emulsion of acetone-extracted bacilli digested for about 18 hours at 37° C. with 10 per cent. trypsin invariably killed mice in doses of 1 mgm. and sometimes in a dose of 0·5 mgm., or even less. If the digestion was carried on for too long a period the solution became less toxic. The symptoms (diarrhœa, &c.) are produced within

half an hour of the inoculation, thus indicating a preformed toxin *in vitro*. Antitoxin prepared by injection of untreated bacteria completely neutralized the toxic properties of the tryptic digest.

It will be seen from the preceding paragraphs that the true nature of the toxins of *B. typhosus* is not yet fully determined. It is difficult to believe that all the various toxic symptoms of typhoid fever can be explained wholly on the basis of anaphylaxis ; it would appear that some specific toxin is at work ; though in some of the so-called ' toxic ' cases of typhoid fever with deep ulceration of the intestine, the toxin may be derived from non-specific sources by absorption from the damaged intestinal wall.

This subject of the endotoxin of various bacteria is one which requires much further work for its elucidation.

One interesting fact is worthy of note, and that is that after prophylactic inoculation of killed *B. typhosus* in man, the spleen has been noted to be enlarged, and as this symptom is almost invariable in typhoid fever it would appear that the lysis of the bacilli in the tissues had given rise to a specific toxin. It is probable that the same effects would be noted in the lymphatic system of the small intestine if the opportunity for observation was available, i.e. the specific toxin of *B. typhosus* has an elective affinity for the lymphatic tissues of the small intestine and for the spleen, just as the toxin of the dysentery bacilli has an elective affinity for the mucous membrane of the large bowel.

Hæmolysins, Cytolysins.

B. typhosus does not secrete a hæmolytic substance as do certain strains of *B. coli*. It is remarkable that when the bacillus is present in the spleen there is little or no reaction in the neighbourhood of the colonies, either cellular or in the nature of a necrosis.

Pathology in Man.

Mode of Spread and Distribution in the Body.

The usual mode of entry of *B. typhosus* in man is by the alimentary tract. If an analogy can be drawn between the rabbit and man the acid barrier of the stomach is one of the main defences against the *B. typhosus*. The bacilli pass it when the stomach is empty and the acid content low, or possibly when the acid is diluted by a large draught of contaminated water.

They thus gain access to the alkaline contents of the upper part of the small intestine and are bathed in bile secreted from the common duct ; here they may multiply to some extent, and it has been stated that at this stage they have been isolated from the fæces, but it is doubtful if *B. typhosus*, at any rate, can multiply in the intestinal contents. If the individual is not immune the bacilli pass rapidly through the wall of the intestine, especially at the lymph follicles and Peyer's patches, and gain access to the lymph spaces, mesenteric lymph glands and the spleen : here they multiply rapidly and at this particular stage of the incubation

period of the disease give rise to vague symptoms of headache and malaise. From these lymph spaces they are carried in the lymph stream to the thoracic duct and thus enter the general circulation. Many are at once destroyed; toxins are set free or elaborated and give rise to the initial stage of the fever. The bacilli are carried by the blood all over the system—to the liver, spleen, bone marrow and gall-bladder wall; from the liver they are carried by the bile to the gall-bladder and to the intestines, where about the end of the first week or beginning of the second they appear in the fæces in large numbers. The bacilli are later on carried in the blood to the kidney and multiply in the pelvis of the kidney and in the urine in the bladder, and are then voided, at about the end of the second week of the fever or the beginning of the third week, in the urine.

Septicæmia.

A septicæmic type of intermittent fever has been described and has been met with in India by the writer, in which there may be no intestinal symptoms whatever, the bacilli gaining entrance to the blood-stream directly by the lymphatics of the tonsils and throat.

Pure septicæmic cases are known to occur in the fœtus of mothers infected with typhoid fever, but such cases in adults are rare and only a probability (Hecht, 1919). For instance, in a case under the care of the writer, blood culture was positive early in an intermittent fever. There were no intestinal symptoms whatever and no abdominal discomfort. Daily examinations of the fæces and urine were made throughout the fever and in convalescence, with negative results. The Widal reaction was very high. Similar cases have been recorded by others, and in some which ended fatally, post-mortem examination revealed a healthy small intestine. It may be said that typhoid fever is a disease in the first instance of the lymphatic system of the small intestine, followed later by a bacillæmia and then by a general infection.

Lesions.

In addition to the blood, urine and fæces, the bacilli may also be readily recovered from the rose spots in the skin. Here again they are present not so much in the capillaries as in the lymphatics. It would appear, as already said, that the typhoid toxin has a special affinity for the closed follicles and lymph structure generally of the small intestine.

Necrosis may be detected in Peyer's patches, but this is due not only to the action of the toxin but also to interference with the blood-supply caused by the remarkable proliferation of the round and endothelial cells; this proliferation is due to the presence of the bacilli and the setting free of their toxins by lysis.

The necrosis is followed by ulceration and inflammation caused by bacteria other than *B. typhosus;* but *B. typhosus* may be set free into the lumen of the intestine by this means.

As the bacilli are carried all over the body in the blood-stream, it is obvious that the lesions to which they may give rise are many, such, for

instance, as pleurisy, peritonitis, periostitis, meningitis, arthritis, pneumonia, endocarditis, phlebitis, nephritis, orchitis, cholecystitis, and gall-stones. The two last are of great interest in view of the importance of the carrier state in man as a means of propagation of the disease. The pathology of this condition has been exhaustively treated by many workers, and this work has been presented in a comprehensive manner by Ledingham and Arkwright (1912) in their book, *The Carrier Problem in Infectious Disease*. Of more recent years Perry (1923) describes the case of a man, aged 43, who gave a history of having suffered from typhoid fever in South Africa in 1900. He was admitted to hospital at Millbank in 1923, with an acute attack of cholecystitis. At operation the gall-bladder was found to be much enlarged and the walls were thickened and fibrosed. Two large gall-stones were removed and in the centre of the stone a nucleus of viscid bile was discovered, and from this *B. typhosus* was recovered in pure culture. Perry describes the histological condition of the gall-bladder as pre-cancerous, which is of interest in view of the next case, described by Henderson (1925). At the post-mortem examination of a woman, aged 79, who had suffered from cachexia and jaundice, a large tumour growth of the gall-bladder with numerous secondaries in the liver was found. In the primary growth about a dozen small faceted gall-stones were discovered, and two of them were washed and cut aseptically; from the cut surface culture was made on an agar slope and from this plate cultures on McConkey's medium. Typical typhoid colonies were obtained. These gave the fermentative reactions of *B. typhosus* and were agglutinated by specific serum to 1/1,000. No typhoid bacilli could be obtained from the intestine. The tumour was an adeno-carcinoma of the gall-bladder.

Ledingham (1926) states that it is generally believed that in the chronic carrier, bacilli are derived from foci in the gall-bladder wall which have been infected by bacilli carried in the blood-stream. He quotes Kwasniewski (1921), who made a careful histological examination of the gall-bladder in 10 fatal cases of typhoid. In three cases he found that the mucosa of the gall-bladder was thrown into shaggy, rugose papillæ, near the free extremities of which were small bacillary nests apparently in close relationship to minute blood-vessels. This description is exactly similar to that of Cummins and Cumming (1914), in their experimental work on rabbits. Surgical removal of the gall-bladder has resulted in cure in the majority of cases, provided there are no necrotic foci in the liver.

Murstad (1921) carried out a post-mortem on a man of 58 who had had typhoid 20 years earlier. *B. typhosus* was isolated from the liver, bile ducts, wall of the gall-bladder and the interior of gall-stones. Murstad considers that the gall-bladder is the home of *B. typhosus* in chronic carriers, the liver only being secondarily invaded. He collected from the literature records of 24 carriers in whom the gall-bladder had been operated upon. Cholecystostomy was done in 8 cases, and 4 were cured. Cholecystectomy was done in 16 cases and 15 were cured. He strongly recommends operation as the only hope of cure.

Another condition peculiar to typhoid is that of producing periosteal nodes on the bones of the hand, the tibia or the ribs. In one such case which came under the personal observation of the Author, the man, who was convalescent about two months, had several such nodes on the metacarpal bones. These nodes were raised and reddened and hard; on puncturing with a hypodermic needle some fluid was obtained, but this proved to be sterile. The patient also had 2 or 3 hard fibrous nodes on the tibia. When playing football some weeks later he was kicked on one of these nodes, which became actively inflamed and suppurated. Some pus was drawn off by means of a syringe and a pure culture of the *B. typhosus* was obtained.

Symptoms.

The chief symptoms of enteric fever are due, in the first instance, to destruction of the bacilli in the blood and tissues, and, secondarily, to the ulceration in the intestine; later in the disease cholecystitis and cystitis may occur: the former being ushered in by rigors, fever and a polynuclear leucocytosis. The blood-count in uncomplicated typhoid fever is usually stated to show a leucopenia with, later, a relative lymphocytosis, but this has been disputed by some recent investigators—Chalier and Morel (1927).

Evidence of Responsibility for the Disease.

That *B. typhosus* is actually responsible for the disease and for many of its complications is proved by the fact that it can be isolated from the blood in the majority of cases (according to Kayser, 100 per cent.) in the first week; and if this should fail it can be isolated later from the fæces, and later again from the urine. Other evidence that incriminates the bacillus is the production of antibodies to it in the blood of patients—agglutinins, opsonins and immune substance can all be demonstrated. In cases of pneumonic typhoid, the bacillus is present in the sputum. It is also found in gall-stones, and it is now generally agreed that gall-stones are caused in a majority of cases by it.

Pure culture infections in man. Further proof that *B. typhosus* gives rise to enteric fever is furnished by the large number of infections which have occurred by the swallowing of pure cultures of the organism either accidentally or intentionally. For instance, Hirschbruch and Forthmann (1919) cite the case of a laboratory assistant who sucked up a culture from a pipette. He had been inoculated three years previously, and he at once washed out his mouth with absolute alcohol, but symptoms developed $13\frac{1}{4}$ days later. They collected no fewer than 57 similar cases from the literature.

Kisskalt is responsible for the statement that old laboratory cultures give rise to more severe infections than fresh cultures. This is contrary to the results of experimental work in laboratory animals.

It is interesting to note that two cases have occurred at the Royal Army Medical College, in the Vaccine Department, in workers who were

employed in preparing vaccines with the old culture (Rawlins). There were no other cases at the time in the neighbourhood ; both cases were severe and both recovered.

Grant (1921) describes an interesting case in which a laboratory assistant sucked up 0·5 c.cm. of a heavy suspension of living *B. typhosus*. His mouth was at once washed out with 50 per cent. alcohol and he was given 0·5 c.cm. vaccine. He had been inoculated 14 months previously. Four days later he complained of headache and malaise, but had no fever and no other symptoms. On the twelfth day after infection *B. typhosus* was present in his fæces and again on the three following days. Blood culture was negative.

A nurse is reported as having swallowed a quantity of the urine of a case of enteric fever, and after an incubation period of 14 days developed fever ; also another nurse deliberately swallowed a pure culture of *B. typhosus* and developed enteric fever of a very severe type, but recovered.

Mechanism of Spread of Infection.

Viability—Action of Light, Heat, Drying, &c.

Much work on this subject has been done, but most of it has been carried out with cultures which have been for some time on artificial media. On the other hand, a certain amount of work has been done with the excreta of carriers actually as passed under natural conditions, and it would appear that the results of such experiments are more likely to give reliable information than those carried out with artificial cultures. The members of the ' Enquiry on Enteric Fever in India ' carried out such a series of experiments (1908). They found that if urine or fæces containing *B. typhosus* is allowed to stand at room temperature (80° F.) protected from the sun, the bacilli very rapidly die out. Urine which contained 60 million bacilli per c.cm. was found to be sterile at the end of 72 hours. Also a sample of fæces from a native suffering from enteric fever which contained *B. typhosus* in practically pure culture was allowed to stand at room temperature protected from the sun, and at the end of 96 hours it was found that the *B. typhosus* had died out. Thin cotton cloth and thick brown blanket were soaked in urine and exposed to direct sunlight. The bacilli were killed in the cotton in two hours, but could be recovered from the blanket for six hours, but not later. The result of these and other experiments are summed up as follows : ' The general trend of the observations shows that the conditions met with outside the human host are not favourable to the prolonged existence of the *B. typhosus*, and therefore the persistence of the disease cannot be explained by any hypothesis that postulates a long extra-corporeal existence of the bacilli '.

Morgan and Harvey (1909) continued this work and extended it. They caused a urinary carrier to micturate on a selected patch of soil. Six hours later *B. typhosus* was easily recovered from the washings of 0·5 gm. of soil, but it could not be recovered after 30 hours ; the soil at that time

was still damp from urine. This experiment was repeated, the carrier micturating on the earth in a corner of a small dark hut. Here the bacillus could be recovered from the soil up to 24 hours, but not later. Very different results were obtained when towelling was soiled with the carrier's urine and allowed to dry, as in this case bacilli could be recovered up to 10 days or more. The probable reason for this is that on towelling there can be no multiplication of *B. coli* or soil bacteria, and hence no rapid destruction of the pathogenic bacteria.

To test the survival of *B. typhosus* in the fæces of a chronic carrier, the following experiment was carried out. The stool was passed on to dry earth in a pail and lightly covered with dry earth. *B. typhosus* was recovered in large numbers for the first two or three days, and it could be recovered from the outer portions of the fæcal mass up to the fifth day, but not later. From the centre of the mass it could be recovered for a fortnight but no longer, even when large pieces of the fæces were emulsified and plated out on several large plates.

In some later experiments (Harvey, 1915) it so happened that a fæcal carrier of *B. typhosus* and a fæcal carrier of *B. paratyphosus* A were available at the same time and a comparative experiment was made. It was found that *B. typhosus* could be recovered more readily and for a longer time than *paratyphosus* A. These experiments were carried out when the temperature was fairly high; but in cold weather or if the fæces were preserved in the ice-chest there was no multiplication of *B. coli*, and *B. typhosus* could be isolated for up to four months.

To compare these results with those obtained when using laboratory cultures in artificial media, the following experiments were carried out. Two loopfuls of 24-hours' broth cultures of *B. typhosus*, *B. paratyphosus* A and *B. coli* were placed as follows:

In 10 c.cm. of peptone water.

Tube 1. Two loopfuls of *B. typhosus* + two loopfuls of *B. coli*.

Tube 2. Two loopfuls of *B. typhosus* + two loopfuls of *B. paratyphosus* A.

Tube 3. Two loopfuls of *B. paratyphosus* A + two loopfuls of *B. coli*.

These peptone tubes were not incubated, but were placed in racks on the bench exposed to daylight but not to the sun. One loopful from each tube plated out with the following results:

10th October, 1909.

Tube 1. *B. typhosus* present; *B. coli* predominant.

Tube 2. *B. typhosus* and *B. paratyphosus* A present.

Tube 3. *B. paratyphosus* A present; *B. coli* predominant.

3rd January, 1910.

Tube 1. Pure culture, *B. coli*; no *B. typhosus*.

Tube 2. *B. typhosus* and *B. paratyphosus* A present.

Tube 3. Pure culture, *B. coli*; no *B. paratyphosus* A.

From these experiments it is obvious that the existence of *B. typhosus* is dependent to a large degree on the conditions of temperature, &c., and whether it is alone or in symbiosis, or, rather, in conjunction with other more robust bacteria. But the fact is that the bacillus can remain alive for months in fæces, and if it gains access to water may be still alive for at least five or six days, and, if the water is at or about freezing point, may remain alive for weeks. At the same time all the evidence is in favour of the view that there is no increase in numbers in such a situation —indeed, the only method by which Harvey was able to show multiplication outside the body was by adding the urine of a carrier to a flask of pasteurized milk. If the urine was contaminated with *B. coli*, or if *B. coli* were already present in the milk, no multiplication could be made out.

Distribution in Nature, Animals and Human Carriers.

In inanimate nature. From the above it is obvious that *B. typhosus* will only be found in Nature where a human carrier or infected person is or has been recently present ; it does not occur otherwise in soil or water or plants or shellfish.

In animals. As previously pointed out, there is no other animal than man which naturally harbours *B. typhosus*, but rabbits, dogs and guinea-pigs become carriers if artificially infected by intravenous injection or direct injection into the gall-bladder, and may remain carriers for several years. In rabbits it is, as a rule, not possible to recover the bacillus from the fæces, so that even as artificial carriers they are not a danger.

In human carriers. The subject of human carriers is such a large one that it is not possible to do more than mention it here, and, as already said, it has been exhaustively treated by Ledingham and Arkwright in their book on *The Carrier Problem*. This brought our knowledge up to the beginning of the War, and not a great deal has been added thereto, although much work has been done. Owing to the size of the problem during the War it was not possible to examine carriers or suspected carriers as often as it is and was possible in less strenuous times.

For instance, in India the members of the ' Inquiry ' (1906–8) examined 86 convalescents practically daily during the fever and well on into convalescence, and 10 of the 86 were found to be excreting bacilli either in the fæces or urine for periods longer than six weeks after the fever had ceased. This would give a percentage of 11·6 carriers ; but, as a matter of fact, only 2 of these men were excreting bacilli more than six months later, and these were the only two that became actual chronic carriers, probably for life. This would give a percentage of 2·3, which is more in line with the figure found by other workers. Both these men were and are still fæcal carriers.

Stokes and Clarke (1915) carried out an investigation of Belgian civilian enteric fever cases, with a view to the discovery of carriers. Eight hundred cases were examined and bacteriological diagnosis was arrived at in 165 cases. *B. typhosus* was isolated from the rose spots in

10 cases. In the search for carriers they employed the brilliant green enrichment method with subsequent plating on Endo's medium. Three months was taken as an arbitrary standard ; and patients if still excreting the bacillus after that time were classed as chronic carriers.

Stokes and Clarke quote Klinger's figures for 604 convalescents, as follows :

Temporary intestinal	70, or 11 per cent.
Temporary urinary	10, or 1·7 per cent.
Chronic intestinal	6, or 1 per cent.

Their own were :

Temporary intestinal	32, or 4 per cent.
Temporary urinary	33, or 4 per cent.
Chronic intestinal	11, or 1·6 per cent.
Chronic urinary	2, or 0·24 per cent.

The chronic carriers were classed as under, and it will be noted that female chronic carriers are three times as numerous as males :

Chronic Carriers

Male, intestinal	3
Female, intestinal	8
Male, urinary	–
Female, urinary	2

The German figures are given as :

Female Carriers	*Male*	*Children*
80 per cent.	18 per cent.	2 per cent.

This preponderance of female carriers, which is invariable, has not so far been explained.

As regards lines of research on the subject of chronic carriers apart from methods of prevention and cure of the carrier-state, it would be a great help in investigating an outbreak if it were possible to identify a particular carrier strain by some special test or reaction.

Ledingham (1926) refers to the German campaign in Alsace-Lorraine in 1914–18. The percentage of carriers varied from 0·3 to 1·6 per cent.

The enteric convalescents during the War from the Western front and from Salonika, Italy, &c., were concentrated in the Enteric Depot at Addington Park. An analysis of the figures shows that 2,808 cases of enteric fever were examined there. It is probable that a considerable number of mild cases did not get as far as the Depot, so that the percentage of carriers found may appear high.

Of the 2,808 cases

546 were typhoid.
837 were paratyphoid A.
1,425 were paratyphoid B.

Still excreting more than six months after :

Total No.	*Carriers*	*Percentage*	*Fæcal*	*Urinary*
T. 546	16	2·93	15	—
A. 837	26	3·1	25	1
B. 1,425	43	3·0	42	1

The ultimate fate of these people as carriers is not known ; the probability is that the majority have now ceased to excrete the bacillus.

Cruickshank (1919) gives an account of work on carriers at Parel, Bombay. One thousand eight hundred and eighty-six cases in all were received ; of these 791 were typhoid, 66 paratyphoid A, 136 paratyphoid B and 326 enteric group. These had been diagnosed in the field by culture or Widal reaction. Each man was examined ten times at 4-day intervals. Forty-nine positives were found : namely, 34 paratyphoid A, 9 typhoid and 6 paratyphoid B ; 47 were fæcal carriers, 2 urinary. After six months only 13 were still carrying, and some were very intermittent. Of these, 8 were paratyphoid A, 4 typhoid and 1 paratyphoid B. All the chronic carriers gave a positive Widal reaction. The carriers were otherwise fit men, but all had at some time complained of pain over the gall-bladder region, with fever. The excretion of bacilli ceased during these attacks. This latter point has also been noted by the writer.

Methods of Transference to Man.

The method of transference of the bacilli is in the majority of instances by contact, and, as a rule, direct or indirect contact with a case of the disease or a carrier. The method of entry is, as already stated, by the alimentary canal, and it has been suggested that the bacilli of the carrier require an intermediary—that is to say, some medium, such as an article of food, in which they can multiply so as to supply the dose necessary to overcome natural or acquired resistance. As already pointed out, milk is an ideal medium for this purpose, especially if it has been sterilized and is subsequently infected.

In the German campaign in Alsace-Lorraine there were 8,042 cases of typhoid. Of these, 4,719 were traced to the source, and it was found that 3,646 were due to infection from pre-existing cases, 722 from carriers, 98 from milk, and 15 due to infection in the laboratory.

Perkins (1922) traced an outbreak of typhoid fever to the contamination of a salad-dressing (cream) by a carrier. Two hundred women were at the lunch, and there were 41 cases of enteric fever. The incubation period was of interest here, as the majority of the cases were at the limits of the period, either the fifth, sixth or ninth day, or eighteenth to twenty-first day. He explains this by dosage (Miner, 1922). Very numerous instances similar to the above can be found in the literature (see Ledingham and Arkwright, 1912).

Tonney and White (1925) studied the viability of *B. typhosus* in oysters in storage. This work arose out of the occurrence of an epidemic of typhoid due to oysters contaminated by infected excreta. They found that the bacillus survived in oyster fluid up to three weeks ; in oysters in their shells kept at a temperature of 70° F. up to eight days, and at 45° F. up to two months. Jordan (1925) was able to recover the bacillus from artificially infected oysters up to 24 days ; there was, however, no evidence of multiplication but some of diminution.

Keyworth (1916) records an interesting outbreak due to consumption of contaminated oysters. Twenty-two people were at a dinner ; 18 ate oysters and 11 of the 18 suffered, 6 with diarrhœa and 5 with continued fever. Three of these were diagnosed as typhoid fever by successful blood culture. None of these three were inoculated ; inoculated people escaped either altogether or else with diarrhœa only.

Natural Resistance.

It used to be generally held that white people were peculiarly liable to enteric fever and that the darker races were immune. This can no longer be said, as during the war the mortality in Indian troops in Mesopotamia was two to three times that of the European, and enteric fever was met with among Chinese, negroes and others in all theatres of the war. This may be explained by changes in diet and environment and close association with other races. It is certainly true that in India or in Africa, enteric is more common among Europeans than among the natives, but to say that the native of Africa or of India is immune to enteric is not correct.

Age. In England the susceptible age is from 17 to 25, in India from 10 to 19 ; it is agreed that the mortality is greatest in young infants and the aged : the mortality of children below 3 years of age being nearly 50 per cent., and about the same in people over 60 years of age.

Local resistance. Infection does not take place through the unbroken skin, but may take place through the mucous membrane of the eye.

A good deal of attention has been given lately, owing to the work of Besredka, to the question of the local resistance of the small intestine. Besredka's (1919) idea is that the resistance to invasion of *B. typhosus* lies in the mucous membrane of the small intestine and not in the stomach. Cornwall, on the contrary, is of opinion that the main barrier—in the rabbit at least—consists in the high acidity of the contents of the stomach. The acidity of the rabbit's stomach contents is much higher than that normally found in man.

Besredka set out to overcome the barrier of the intestine and to enable the bacilli to penetrate the mucous membrane. His experiments show that the administration of ox-bile to rabbits lowers their resistance to the invasion of *B. typhosus*—that is to say, they can, after administration of bile, actually be infected by the buccal route and

smaller doses are required to kill when the dose is given by the intravenous route than in unsensitized animals.

Besredka considers that this lowered resistance is due to a mechanical stripping of the mucous membrane of the small intestine, but Cornwall does not agree with this view, as he administered bile to rabbits, killed them and on examination could find no evidence either by macroscopical or microscopical examination of any extensive stripping of the mucous membranes. He also points out that with *B. typhosus* the minimum lethal dose for rabbits by the intravenous route is exceedingly difficult to arrive at, and if a series of rabbits is inoculated the dose may vary widely and does so vary from day to day. Besredka himself also remarks on the difficulty of obtaining a standard lethal dose.

In Besredka's rabbits, if a sufficient dose is given by mouth following administration of ox-bile, an incubation period of about four days is followed by fever, diarrhœa and death in about six days. *Post mortem*, intensive congestion of the small bowel is found with swelling of Peyer's patches ; the contents of the bowel are fluid and on culture are found to be swarming with *B. typhosus*. Only a few bacilli are found in the large intestine, and Cornwall states that he was never able to find them in the rabbits' fæces during life.

One experiment of Besredka's is cited to show the effects on absorption through the intestine following a dose of bile. At 5 o'clock in the evening rabbits were given a dose of 10 c.cm. of bile by the mouth ; they were kept without food until the following morning. At 10 o'clock another dose of 10 c.cm. of bile was given, and two hours later the dose of culture, either *per os* or by the intravenous route. By this means rabbits were infected *per os*, and when the dose was given intravenously, 1/100 of an agar slant was sufficient to kill, as compared with 1/10 of a culture required in unsensitized rabbits.

Veratti and Cattaneo have shown experimentally that bile does not strip the intestine of rabbits of its lining epithelium, but it does sensitize rabbits to oral infection, this is possibly due to the fact that bile in some way neutralizes the acidity of the stomach contents and provides a nidus for the growth of *B. typhosus* in the small intestine and also causes a flow of normal bile from the gall-bladder. It has been shown by Pannett and Wilson (1921) that small doses of bile salts added to a test meal cause the rapid clearance of the stomach contents ; and Mellanby also has found that bile causes rapid absorption through the intestinal wall into the lacteals. This action of bile, however, would not explain the fact that after exhibition of bile a smaller dose given intravenously kills.

Cornwall also showed that if bacilli are introduced directly into the jejunum they rapidly traverse the wall of the intestine and are found in the general circulation within one hour, without administration of bile.

This question of the local resistance of the intestine will be further discussed under ' Prophylaxis '.

Immunization and Therapy.

Production of Relative or Solid Immunity in Experimental Animals.

It has already been mentioned that in the very earliest experimental work with *B. typhosus*, it was noticed that if animals received a sublethal dose they were thereafter more resistant to subsequent doses than control animals.

Various methods have been used for the immunization of animals by neutralizing the toxic substance by formalin, or by sensitizing the bacilli by contact with immune serum. Whatever method has been used, it has, as a rule, been found that immunity can be established in animals by subcutaneous or intravenous injection of killed cultures, but that this immunity is in no way absolute or solid and can be overcome by massive doses of living bacilli; indeed, in the process of immunization many animals may be killed if the dosage is too large or is given at wrong intervals.

Besredka's oral vaccine. Interesting work of recent years is that of Besredka on the immunization of animals by the oral route. The drawback to all previous work on experimental animals, so far as it had reference to vaccination in man, was that no animal was available which could be infected by the mouth as man is, and, therefore, although we could say that subcutaneous inoculation of killed vaccine of *B. typhosus* rendered an animal immune, or at least relatively immune, to the injection of further test doses of the same bacillus, we could not definitely argue from that that subcutaneous inoculation in men would protect them from infective doses by the mouth. Besredka (1919) showed that living bacilli given by the mouth did not in any way protect rabbits from a lethal dose of *B. typhosus* given later into the vein ; but if the protecting dose given by the mouth was preceded by a dose of bile a solid immunity was produced. Control rabbits without the previous immunization by the mouth died in three days after the test dose.

Agglutinins appear in the blood of animals immunized by the mouth and may reach as high a titre as 1/10,000 or 1/20,000, at about the twenty-fifth day after the dose has been administered ; but the interesting point is that although these antibodies disappear from the blood, or at least can no longer be demonstrated, the immunity remains and further doses of virus by the mouth do not raise the agglutination titre. Besredka looks upon agglutinins not as a measure of immunity but as a concomitant, phenomenon in the process of immunization. An animal may be solidly vaccinated against typhoid and yet show no agglutinins in its blood. He also states that immunized rabbits if given a dose of living bacilli by the mouth will produce agglutinins in the blood, and yet can be proved not to be immune to test doses. (The last statement would suggest that there is absorption through the unsensitized intestinal wall, which is contrary to his previous contention.)

Cornwall (1926) and Gye (1923) gave doses of *B. typhosus*, killed and living, *per os* to many rabbits, some with bile and some without, and afterwards tested with intravenous doses; no immunity was shown; the same results were obtained as in unsensitized controls.

Prophylactic Immunization in Man.

It was natural that as soon as it had been noted that immunity was produced in animals by subcutaneous doses of killed bacilli, it should be suggested that this procedure might be employed in man.

At first, the figures obtained as a result of the use of these vaccines in man were not convincing, but the case mortality rate in the South African War showed a remarkable reduction among the inoculated men:

1,758 inoculated	142 deaths	8 per cent.
10,980 uninoculated	1,800 deaths	16·6 per cent.

Yet the fact that 142 persons who had been recently inoculated died of enteric fever shows that, although there was evidence of protection, it was far from being a complete immunity.

The vaccine used has been altered in one or two particulars from time to time. Statistics regarding its use are now world wide, and are practically uniform in their general trend, which is that there is overwhelming evidence of protection, although the protection afforded is not complete.

Besredka, in introducing his oral method of vaccination, points out that, so far, cases do occur in inoculated men whatever vaccine has been used—lipo-vaccine, heat killed, formalinized or sensitized—whereas cases are exceedingly rare in men who have had enteric fever previously. In one fever hospital during the war he found 300 men who had had enteric fever previously. The blood cultures of all these were positive, but they were all paratyphoid cases. Of 102 cases from whose blood *B. typhosus* had been isolated, 28 had never been inoculated, 38 were insufficiently inoculated—1, 2 or 3 injections only, 41 were fully inoculated—4 doses.

Whether Besredka's oral vaccine can confer a solid immunity can only be determined by trial on a large scale in the field.

Vaillant (1922) gives the following figures: among 1,386 men vaccinated *per os* there were 5 cases, or 0·17 per cent.; among 173 men vaccinated subcutaneously there were 4 cases or 2·3 per cent.; and among 600 unvaccinated men there were 21 cases or 7·7 per cent.

Here again we see that some cases did occur among the men vaccinated *per os*, although fewer than in the unvaccinated; otherwise the figures are not of much value.

Before the war, the troops on home service in Great Britain were not inoculated, but practically all those serving in India were protected by inoculation. This measure had been re-introduced in 1906 and was pushed gradually, so that by 1909 practically 100 per cent. of the troops in India were protected, and the result was that enteric fever in that

country had, even before the war, ceased to be a menace to the health of our troops (see Table I).

TABLE I.

All India. Enteric Fever.

Year	*Ratio per 1,000 Admissions*	*Deaths*
1909	8·9	1·58
1910	4·6	0·63
1911	3·8	0·33
1912	2·6	0·39
1913	2·3	0·25

	Peshawar Garrison. Admission rate per 1,000
1909	11·3
1910	7·9
1911	3·0
1912	0·6
1913	0·6
Meerut.	
1909	26·0
1910	2·3
1911	1·8
1912	3·2
1913	1·7

The more favourable situation of the Armies in France, as regards diagnostic facilities and their freedom from any epidemics of such a size as to hamper the work of the laboratories, enabled accurate information to be accumulated on a scale to be worth analysis in connection with most points of scientific interest.

The tables which follow (II to VIII) are taken from the 'Official History of the War'; Medical Services; Pathology (1923). The figures were collected by the late Sir William Leishman (1921) and are probably the most complete records obtainable in any country and in any language.

When war broke out none of the troops composing the Expeditionary Force were protected against enteric fever by inoculation, and there was, in many instances, little time to carry this out, the result being that the majority of the men of the original Expeditionary Force were not at first protected. Some 30 per cent. had, however, received a single dose of vaccine (which at the time contained only *B. typhosus*); the dose employed was 1 c.cm. (1,000 million *B. typhosus*), and this conferred a partial protection. At no time during the war was inoculation compulsory, yet by the end of 1914 some 80 per cent. of the men in France had been inoculated with the single *B. typhosus* vaccine and the percentage of men inoculated rose gradually until by the end of 1915 over 90 per cent. were protected.

In view of the prevalence of paratyphoid fevers, especially paratyphoid B, in France and elsewhere, it was decided during 1915 that paratyphoid A and B should be added to the original vaccine. This was accordingly arranged for, and the new T.A.B. vaccine was first issued for use in January, 1916. This vaccine, as used by the British troops from 1916 onwards, was an emulsion in saline of the bacilli killed by heat at a temperature of 53° C. to which lysol was added in a strength of 0·3 per cent. The strength of the vaccine in 1 c.cm. was :

B. typhosus	1,000 million.
B. paratyphosus A	750 million.
B. paratyphosus B	750 million.

The dose recommended was 0·5 c.cm., and, 10 to 14 days later, 1 c.cm.

Fifty million c.cm. of vaccine were issued from the Royal Army Medical College, Millbank, and inoculated into several millions of men, and not a single case was reported of any accident attributable to want of care in the preparation of the vaccine.

Table II deals with the total cases of typhoid and paratyphoid fever in the British Expeditionary Force in France, during the year August, 1914, to August, 1915.

TABLE II.

Total Cases and Case Mortality from Typhoid and Paratyphoid, British Expeditionary Force, August, 1914, *to 9th August,* 1915.

	Cases	Deaths	Case mortality
Typhoid.			
Not inoculated T.V. within 2 years ..	545	111	20·36
Inoculated 1 dose T.V. within 1 year ..	190	13	6·84
Inoculated 2 doses T.V. within 2 years ..	236	19	8·05
Totals	971	143	Av.14·72
Paratyphoid.			
Not inoculated T.V. within 2 years ..	113	4	3·53
Inoculated 1 dose T.V. within 1 year ..	183	3	1·63
Inoculated 2 doses T.V. within 2 years ..	423	8	1·89
Totals	719	15	Av. 2·08
Enteric Group.			
Not inoculated T.V. within 2 years ..	24	—	—
Inoculated 1 dose within 1 year	27	—	—
Inoculated 2 doses T.V. within 2 years ..	43	—	—
Totals	94	—	—

As has been mentioned, during this period the men were only protected by the single typhoid vaccine (T.V.) as T.A.B. vaccine was not introduced in France until January, 1916. It will, therefore, be understood that only protection against true typhoid had so far been attempted. As a result of research it had been decided that men who had received a single dose

T.V.1. vaccine, i.e. 1 c.cm. containing 1,000 million *B. typhosus*, should be considered as protected for one year; those who had received two doses T.V.2 of 0·5 c.cm. and 1 c.cm., a total of 1,500 million *B. typhosus*, as protected for two years.

All particulars as regards inoculation were obtained from the soldier's pay book, which he carried on his person, and the entries were made therein by the medical officer carrying out the inoculation.

In Table II the striking point seen is the difference between the case-mortality in the non-inoculated and inoculated groups —20·36 and 7·44 respectively.

The fact that typhoid inoculation modifies the attack of enteric fever, if the inoculated person should subsequently contract the disease, was clearly established during the war. The experience one had in India in pre-inoculation days, when severe unmodified and fatal cases of enteric were commonly seen, was in marked contrast to the mild cases met with in France among the inoculated men. Sir William Leishman remarked that in the early stages of the war severe toxic and fatal cases did occur among the uninoculated, but they were not met with later on when inoculation was universal, except in one instance, and this was among the members of a Labour battalion who had escaped inoculation.

An interesting point in this connection also emerged from the figures (see Table VIII), namely, that successful blood culture was more difficult to obtain among the inoculated cases than among the uninoculated, showing that the bacillæmia was less intense and of shorter duration in the former class of case.

The statement that enteric in inoculated individuals takes a particularly mild form has been called in question by Vaughan (1920), Ledingham (1921) and others, who maintain that comparative analyses of clinical syndrome or of mortality statistics in inoculated and uninoculated individuals must be based on culturally diagnosed series.

Table III gives the figures for the period August, 1914, to the end of January, 1915, and includes the original Expeditionary Force, of whom only approximately 30 per cent. were inoculated and mostly with the single dose. During this period the inoculation state gradually increased to 80 per cent. of the Force.

TABLE III.

Total Cases and Case Mortality from Typhoid Fever, British Expeditionary Force, August, 1914, to 29th January, 1915.

	Cases	Deaths	Case mortality
Not inoculated within 2 years	305	34	11·1
Inoculated 1 dose T.V. within 1 year ..	83	1	1·2
Inoculated 2 doses T.V. within 3 years ..	33	—	—
Totals	421	35	Av. 8·3

Precise information on the inoculation strength was not possible to collect for military reasons, so the incidence per 1,000 is not given here as in some subsequent tables. All the same, the contrast in the case mortality is dramatic, as is the fact that only one death from typhoid occurred in this period in an inoculated man.

Table IV shows the incidence of typhoid and paratyphoid during the year 1915 among the inoculated and non-inoculated, and is one of great importance, since it deals with a period during which T.V. only was in use, and in which full information was available both in respect of the percentage of inoculation in the Force and of the mean strength of the Armies. From this information it has been possible to obtain a substantially accurate view of the relative incidence of these fevers in the two groups, and thus to arrive at a statistical evaluation of the protective effects of inoculation.

TABLE IV.

Monthly Rates of the Incidence of Typhoid Fever among the Inoculated and non-Inoculated during 1915.

	Percentage of inoculated	Cases per 10,000		
		Amongst inoculated	Amongst non-inoculated	Amongst all troops
January	80·0	2·98	9·93	4·37
February	88·3	1·85	17·76	3·71
March	92·0	1·41	21·33	3·01
April	93·0	0·8	8·59	1·35
May	94·0	1·1	8·46	1·54
June	95·0	1·84	2·74	1·13
July	95·8	1·0	2·57	1·66
August	97·1	0·89	4·51	0·99
September	97·5	0·49	4·1	0·58
October	97·5	0·41	1·72	0·44
November	98·1	0·28	2·27	0·32
December	98·5	0·23	5·53	0·31
For the year ..	—	9·5	103·52	13·78

It will be noted that for this year—1915—when the men were only inoculated with T.V. vaccine, the incidence of true typhoid among the inoculated was 9·5 cases per 10,000, whereas among the uninoculated the ratio was 103·52 per 10,000 men.

Among men who were inoculated only against true enteric fever (*B. typhosus*) with T.V. vaccine and afterwards contracted paratyphoid fever, the reduction in mortality was almost exactly the same as in those who

were inoculated with T.A.B. vaccine and later contracted paratyphoid fever. It is also evident from the figures that fewer men contracted paratyphoid fever among those inoculated with T.V. vaccine only than among those who were totally unprotected.

TABLE V.

Annual Rates of the Incidence of Typhoid, Paratyphoid and Enteric Group among the Inoculated and Non-Inoculated (with T.V.) for 1915.

		Rates per 10,000			
		1. Typhoid	2. Para-typhoid fevers	3. Enteric group	1, 2 & 3
Inoculated	614,715	9·5	21·53	2·87	33·91
Non-inoculated ..	29,365	103·52	39·84	7·49	150·85
Total	644,080	13·78	22·37	3·08	39·24

Indeed, the general impression was that the simple typhoid vaccine provided a certain amount of group protection against paratyphoid fever. This was also evidenced by the fact that the incidence of paratyphoid fever had already begun to fall in France before the introduction of T.A.B. vaccine.

In 1917 only 1,275 cases of enteric fever were diagnosed as contrasted with 2,760 in 1916, i.e. less than half, although the strength of the Force had been approximately doubled in 1917 ; by this time practically 95 per cent. of the men were fully protected with the T.A.B. vaccine.

Another interesting point brought out by a study of the monthly sick rate during these years was that in periods of ' activity ', when it was not possible to carry out the usual meticulous sanitary arrangements, there was invariably a sudden and alarming rise in the amount of bowel complaints, but these were bacillary dysentery, diarrhœa, &c., not enteric fevers ; the obvious conclusion being that the men were protected against enteric fevers by means of the specific inoculation, whereas they were not so protected against dysentery and diarrhœa. It must be obvious that but for this special protection each of these periods of activity would have been followed by an outbreak of enteric fever with much loss of life and efficiency.

In 1918 the total number of cases of enteric fever fell to 376, and this in spite of mobile warfare and of a rise in strength to two and a half million men.

TABLE VI.

Total Cases of Enteric Fever in the British Armies in France, from the Commencement of Operations in August, 1914, *to* 31*st December,* 1918.

	Cases	Deaths	Case mortality, per cent.
1. *Typhoid.*			
Inoculated T.V. or T.A.B. vaccine ..	1,728	79	4·57
Non-inoculated T.V. or T.A.B. vaccine ..	703	129	18·35
Total	2,431	208	8·55
2. *Paratyphoid.*			
(*a*) Inoculated T.A.B. vaccine	1,357	17	1·25
Non-inoculated T.A.B. vaccine ..	2,694	35	1·34
Total	4,051	52	1·28
(*b*) Number of cases from the commencement of operations in men uninoculated with either T.V. or T.A.B. :			
Paratyphoid A	74	3	4·05
Paratyphoid B	251	8	3·18
Total	325	11	3·38
(*c*) Number of cases which occurred subsequent to the introduction of T.A.B. vaccine in France, on 1st February, 1916 :			
(i) *Paratyphoid A.* Inoculated with T.A.B. vaccine ..	463	5	1·08
Paratyphoid B. Inoculated with T.A.B. vaccine ..	894	12	1·34
Total	1,357	17	1·25
(ii) *Paratyphoid A.* Non-inoculated T.A.B. vaccine ..	265	3	1·13
Paratyphoid B. Non-inoculated T.A.B. vaccine ..	858	9	1·04
Total	1,123	12	1·06
3. *Enteric Group* (i.e. clinical diagnosis ; bacteriological evidence insufficient to distinguish with certainty between typhoid and paratyphoid fever) :			
Inoculated T.V. or T.A.B. vaccine ..	878	4	0·45
Non-inoculated T.V. or T.A.B. vaccine	63	2	3·17
Total	941	6	0·63

Note. In all instances the ' non-inoculated ' includes men who had received a single dose of vaccine more than a year before the attack, or two doses more than two years before.

TABLE VII.

The Relative Incidence and Mortality from the Enteric Fevers among the British Troops engaged, respectively, in the South African War and in the war of 1914–18.

	Total cases	Total deaths	Case mortality per cent.	Mean annual strength	Annual incidence per 1,000 of strength	Annual death rate per 1,000 of strength
South African War, 1899–1902 ..	57,684	8,022	13·9	208,226	105·00	14·6
The Great War, 1914–18	20,139	1,191	5·9	2,000,000	2·35	0·139

TABLE VIII.

The Results of Cultivations from the Blood and Excreta of Enteric Fevers in the Armies in France, August, 1914, *to December,* 1918.

	Inoculated					Non-inoculated				
	Cases	Isolations. All sources		Isolations from blood		Cases	Isolations. All sources		Isolations from blood	
		Total	Per-centage	Total	Per-centage		Total	Per-centage	Total	Per-centage
Typhoid	1,553	416	26·7	252	16·2	578	254	43·9	218	37·7
Paratyphoid A ..	438	66	15·0	18	4·1	554	240	43·3	150	27·0
Paratyphoid B ..	863	375	43·4	58	6·7	1,953	967	49·5	387	19·8
Total T + A + B	2,854	857	30·0	328	11·4	3,085	1,461	47·3	755	24·4

Total cases analysed .. .. .. 5,939.
Total isolations .. .. 2,318 = 39 per cent.
Total isolations from blood .. .. 1,083 = 18·2 per cent.

Pfeiffer (1922) gives the following figures for inoculation in the German Army :

Non-inoculated .. .. Mortality, 12 to 20 per cent.
Inoculated .. .. Mortality, 2 to 3 per cent.

In support of the contention that blood culture is less likely to be successful in inoculated cases, as is shown in Table VIII, Just (1916)

gives some interesting figures which also bear on the question of the degree of protection which typhoid vaccine appears to give against paratyphoid infection. In unprotected men suffering from typhoid fever the percentage of successful isolation of *B. typhosus* from the blood was 72 ; the percentage in partially protected was 50 ; and in fully protected 56. In cases infected with paratyphoid A the percentage of successful isolations from the blood was 100, whereas in people protected with *B. typhosus* vaccine it was only just over 50 per cent.

Ledingham (1920) brings out the interesting fact that the percentage of successful isolations from the blood was considerably higher in Indian troops in Mesopotamia than in British. This he correlates with the fact that the mortality also was greater—2 to 3 times—i.e. the disease ran a more severe course in the Indian than in the British soldier.

The percentage of inoculations among the British soldiers generally was much higher than among the Indian, but there are no figures available to show whether the actual cases among the Indians were inoculated or not.

Further proof of the efficacy of typhoid vaccine has been provided by the evidence from various countries (France, America, Germany) that the incidence of enteric fever on the sexes has become entirely altered since the war. Thus Chauffard, Achard and Sergent (1921) record a practical disappearance of typhoid fever in France among males between the ages of 20 and 45. In ordinary times the male rate is higher than the female, but during the years 1918–19–20, the female rate for this age group was much higher than the male. This drop in the typhoid incidence in men of that age group they consider could only be due to immunization in the Army. The typhoid death rate corroborated this inference. The same fact was recorded in America in the annual *résumé* on enteric fever in the ' Journal of the American Medical Association '.

Hahn (1927), in connection with the recent epidemic in Hanover, where there were 2,423 cases of enteric, found that between the ages of 10 and 30 males and females were equally affected, but between ages 31 and 50 females were much more affected than males, and the mortality was twice as high among women as among men, many of whom had been inoculated during the war. If this is so, then the duration of immunity after inoculation must be longer than is generally supposed. At any rate, increased agglutinins have been found in the blood of inoculated persons up to five years after inoculation. Such agglutinins were of the ' floccular ' type.

In comparing or estimating the efficacy of bacterial vaccines when inoculated into animals the principal indication previously has been the agglutination titre. It is true that frequently the opsonic index and the bactericidal content of the serum and complement-fixation tests have also been considered, but the principal reliance has been placed on the presence of agglutination. The reason for this was that the agglutination titre was easily determined and, as a rule, the other antibodies followed a similar curve.

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It will be gathered from what has already been said that there is considerable difference of opinion whether the agglutination curve does reveal the immunity of the inoculated man or animal, and indeed, it is an established fact that persons who have had enteric fever may lose, and generally do lose, all agglutinins from the serum, and yet rarely or ever do they again contract enteric fever ; also persons showing a high agglutinin content in the serum at the end of an attack of fever may and do suffer from relapse. Besredka also has shown that agglutinins are not necessarily a guide to the degree of immunity, although they may on occasions run parallel to it.

Felix and Olitzki (1926) find that ' small flaking ' immune sera possess more bactericidal power than the ' large flaking ' sera. Indeed, if from a serum which contains both types of agglutinins the ' small flaking ' are removed by absorption, the bactericidal power of the serum is entirely lost. Burnet (1924) has demonstrated that in cases of typhoid fever which approximate clinically to the normal type of the disease, the serum shows an almost equal development of agglutinins to the two antigenic constituents of the typhoid bacillus, i.e. floccular and granular, or flagellar and somatic, whereas the agglutinins produced in the serum of inoculated persons are almost entirely floccular.

Dawson (1915) was the first to note that the sera of inoculated men did not agglutinate a culture of a Gaertner strain whereas the sera of non-inoculated enteric fever cases did, and he suggested this as a method for differentiating between inoculation and infection agglutinins.

Felix (1924) associates the fine granular type of agglutination—somatic agglutination—*with immune body*, and as typhoid vaccines in his hands did not produce this type of agglutination in man he considers, therefore, that our present vaccines are not likely to give good results so far as protection is considered.

Arkwright (1927) carried out some experiments on the protective power of rough variants of typhoid and paratyphoid bacilli. With *B. paratyphosus* A, he found that vaccines prepared from ' smooth ' cultures protected against a ' smooth ' test dose, whereas a ' rough ' vaccine did not so protect. The same was true to a lesser extent with *B. typhosus*. He also found that heating a ' smooth ' vaccine to 100° C. did not destroy its protective properties, although it removed or destroyed the flagellar antigens. From the results of these experiments he considers that the heat-stable antigen is the antigen concerned in the production of immunity, and that the floccular antigen or agglutinin, on which most of the previous work on vaccines has been based, is not of the same significance. He suggests that non-virulent, spontaneously-agglutinating cultures should not be used for the preparation of typhoid vaccines, and that the best protection would be given by large doses of ' smooth ' boiled vaccine.

Although it is not likely that anyone would use for the preparation of a vaccine, a culture which spontaneously agglutinated in salt solution, yet the greatest care should be taken to select smooth strains. On at

least three occasions during the war, the whole ' brew ' of typhoid vaccine had to be discarded at the Royal Army Medical College as it spontaneously clumped when suspended in saline ; but on replating and picking colonies the culture regained its ordinary behaviour in saline and did not again ' clump ' for some months.

It is possible that certain failures to protect, in groups of men inoculated at the same time, may have been due to the preparation of a vaccine from a ' rough ' culture.

Hofmeier (1927) found that both labile and stable antigens in *B. typhosus* are able to fix complement in the presence of the corresponding antibody, which is in contradiction to the opinion of Felix.

So far as *B. typhosus* at least is concerned, it would appear to be unnecessary to consider a qualitative receptor analysis, when a more simple explanation, such as that suggested by Orcutt, Tulloch and others, and originally by Smith and TenBroeck, is available. It has been shown that by the use of the centrifuge we can separate the flagella from the bodies of the bacilli, and that these two components represent two antigens. It is reasonable to expect that the flagellar antigen is the simpler one, whereas the somatic is of a more complex nature, and when injected into animals gives rise to the production of immune bodies apart from agglutinins. It does not, however, follow that the flagellar portion is entirely without use in vaccine preparation : the motility of the typhoid bacillus may not be without its significance in the invasion of the human anatomy *per vias naturales*. Thus, if a flagellar antibody is present it may conceivably be of use in checking invasion ; for these reasons it would seem reasonable to utilize the complete bacillus and in as undamaged a state as possible, as has been the practice in the past. In this connection a recent paper by Springut (1927) is of interest as it goes to show that in a vaccine for ' Mouse Typhoid ', the heat-labile antigen is of importance for protecting against feeding with living bacilli.

Whitehead (1927) has recently reviewed the work done on this subject and has carried out some experiments with the stock strain which has been used for many years to prepare the Army vaccine. This same strain has been also used in America for vaccine preparation, and wherever it has been used the statistical results have been favourable. From his review of the work of Arkwright and Felix two provisos are suggested : (1) that prophylactic vaccines should be made from the smooth form of the organism ; (2) that the blood of properly protected persons should agglutinate in a granular as well as a floccular manner, thus denoting the presence of heat-stable antigens and agglutinins.

Whitehead compared the ' Rawlins ' strain with a known smooth strain supplied by Dr. Arkwright. The ' Rawlins ' strain did not auto-agglutinate in normal saline solution, although the colonies were distinctly rougher than those of the smooth strain and there was some deposit in broth. Three rabbits were inoculated with ' Rawlins ' vaccine and three with vaccine prepared from the known smooth strain. All six rabbits showed

granular agglutinations for the smooth strain up to a titre of 1/2,500 ; the floccular agglutination titre was 1/12,500 ; the granular agglutination was tested by means of cultures of the smooth strain from which the flagellar antigen had been removed by treating the organisms with absolute alcohol for 24 hours. Whitehead concludes that the ' Rawlins ' strain may still be considered a smooth strain in spite of the length of time that has elapsed since its isolation and its frequent subculture. A possible reason for this is that it has always been the custom to carry on the vaccine strain by subculture from a single colony, and, naturally, smooth normal colonies would be selected, and any culture which sedimented in broth, or spontaneously agglutinated in saline, would be discarded. Numerous attempts have been made recently to get rough colonies from this strain, but so far without success ; apparently it has become ' fixed ' somewhere between rough and smooth so far as colony variation is concerned.

Whitehead also tested the serum of the following for the presence of granular agglutinins : (1) experimental animals inoculated with ' Rawlins ' and S ; (2) inoculated persons inoculated with ' Rawlins ' only ; (3) convalescents from enteric fever.

Class 1 showed the presence of granular agglutinins in large amount in the serum. Of Class 2 only one person showed such granular agglutinins ; he was a recently inoculated man. None of the convalescents showed any granular agglutinins in the serum. The difference in the first two cases might be explained by the difference in size of dose per kgm. of weight, but this would not explain the absence of granular agglutinins in the serum of convalescents, who must, presumably, have had a maximum dose.

Although such remarkably successful results have been obtained by the vaccine at present in use, no one can say that it gives complete protection, or that it gives as good protection as is possible. There must, therefore, be room for improvement, and in this direction lies one of the most fruitful fields for investigation and research.

Therapeutic Vaccination.

Specific. Non-specific. Although the treatment of typhoid fever by means of autogenous or stock vaccines has been employed for more than twenty years, it is still difficult to arrive at a definite conclusion as to its efficacy. The reason for this lies in the fact, already alluded to, that enteric fever varies so markedly in its severity ; in some epidemics all cases may be mild, in another all may be severe, and more commonly the severity of the disease in the same outbreak varies remarkably from the severe toxic to the mild ambulatory case. It is, therefore, extremely difficult to say what effect the treatment by vaccines has had in any particular series of cases. At the same time it would appear from a survey of reports on the subject and from the personal experience of the writer that, on the whole, this method of treatment is of distinct value provided that it is carefully and scientifically carried out.

During the war it was at first used to a considerable extent, but was later abandoned in great part as not yielding satisfactory results. For example, Whittington (1916) gives his experience in the treatment of over 200 cases. He at first had been inclined to attribute good results to the method, but after studying a large number of untreated cases he arrived at the conclusion that the variety of type of the disease was so diverse that it was not possible to give an opinion on the subject ; but his final conclusion, after treating 230 cases, was that the method could not be recommended. On the other hand, Besredka and Gay (1917) treated about 100 cases with a polyvalent sensitized typhoid vaccine sediment given intravenously in doses of 1/50 mgm. and with excellent results ; the mortality was reduced to 6·6 per cent. Stein (1919) treated over 500 cases with Besredka's sensitized vaccine. He found that it shortened the duration of the disease in the majority of the cases (84 per cent.) and its use was not attended by any risks. The mortality in his series of cases also worked out at 6 per cent. He preferred the subcutaneous or intramuscular route to the intravenous ; 0·5 c.cm. doses containing 250 million bacilli were employed, and four injections were given. Melnotte and Farjot (1927) treated 100 cases of typhoid fever by means of a stock vaccine given by the mouth in daily doses of 800 million bacilli. Although the duration of the fever was not affected, the treated cases were much milder than the untreated and showed a mortality of 6 per cent., as compared with 26 per cent. in a previous series of untreated cases.

It is necessary to differentiate between the two actions of typhoid vaccine given intravenously ; the one is immediate and non-specific, and is in the nature of a protein shock reaction : a similar reaction may also be produced by the injection of sterilized milk or solution of peptone ; the other is a much later action, only commencing four or five days after the injection ; it results in the actual formation of specific antibodies by the tissue cells. The immediate reaction is signalized by a rise of temperature and a leucocytosis, and is followed by a rapid fall of temperature, often to below normal ; the temperature may not rise again above the normal line or may rise only slightly, and the fever may cease in a few days.

This rapid fall of temperature following immediately after a dose of vaccine and occasional disappearance of the fever has been attributed to a sudden mobilization of preformed specific antibodies from the tissue and polynuclear cells. But as the same effect may be caused by heterologous proteins, it cannot be said to be a specific reaction, as is the later appearance of antibodies in the blood, which is a specific response to the antigen employed.

Recent work on the necessity for selection of cultures for prophylactic vaccines, shows also, and with even greater force, that such selection is required in the preparation of therapeutic vaccines. In this connection the method of heating to 100° C. might be usefully employed, with a view to rendering the vaccine less toxic.

Sera. Sera have also been employed in the treatment of the disease, but the results have not been of the happiest, although success has been claimed by Étienne (1916) and Pezzi (1922).

Smith (1924) gives an account of a series of cases treated with doses of typhoid bacteriophage: 5 gm. of fæces from a normal person were emulsified in 50 c.cm. of broth (pH 8·0) and incubated overnight. The broth was then filtered, and 2 c.cm. of filtrate added to 10 c.cm. of broth. Sufficient of a young culture of *B. typhosus* was then emulsified in this broth to give an opacity equal to 250 million per c.cm., incubated, and subsequently cultured on agar. The activity of phage was judged by lytic areas on the surface of the tube. By a process of passage the activity of the phage was exalted. Several cases were treated by doses of 0·5 c.cm. phage subcutaneously and 2 c.cm. by the mouth. It appeared that the phage caused the bacillus to disappear from the fæces, but had no effect on the bacteria in the urine.

Treatment of Carriers.

Vaccine treatment has been given a very thorough trial by a number of workers, including the writer, but it is difficult to say whether there have been any successful results.

A great variety of drugs have been used in attempts to eradicate the carrier state, either urinary or fæcal, but without much success. Conradi employed chloroform *per os* and *per rectum* and claims that he had satisfactory results. The following substances have been tried in animals by various experimenters (Hailer and Wolf, 1914): butylchloral-hydrate, metaxylenol, symmetrical xylenol *per os*; pyrogallol, beta naphthol, sod. sal. intravenously. In man, urotropine has been tried without success (Morgan and Harvey, 1909), though it is possible that very prolonged treatment might give better results.

In view of the failure of such methods in the majority of cases, the advisability of cholecystectomy should be considered, although even this radical method has not invariably been successful; but the presence of gall-stones is certainly an indication for operation.

Antibacterial Measures.

Disinfection, Heat, Chemicals, &c.

The ' naked ' typhoid bacillus is peculiarly vulnerable to such destructive agents as heat, chemicals and antiseptics, but, as has been already shown, is exceedingly difficult to eradicate from its nidus in the gall-bladder, spleen, bone marrow or kidney of the human carrier, and here operation may be the only possible cure.

B. typhosus can be killed at a temperature of 60° C. if exposed continuously for half an hour, and the majority of the bacilli are killed when exposed to a temperature of 53° C. for one hour; when exposed momentarily at 80° C. they are all immediately killed.

Perchloride of mercury is particularly lethal to the naked bacillus, but has little action on the bacillus in the fæces.

Cresol is useful when the pail system of sewage disposal is in use, because although it may not, when diluted by urine as it must be, be capable of killing bacteria in fæcal matter, yet it is peculiarly repellent to flies.

The action of sunlight has already been discussed.

Prevention of Dissemination by Flies.

As a means of the carriage of small quantities of infected excreta from the latrine to articles of food, the fly is a most efficient agent. The bacteria may be carried in two ways: (1) on its legs, (2) in its intestine. The bacilli may be either rubbed off on to the food or milk or else regurgitated thereon or passed in the excreta. Experiments have shown that the life of the bacillus in the intestine of the fly is not a prolonged one: they are, indeed, rapidly destroyed there; but they exist certainly long enough to be carried from the latrine to the kitchen if these two are in close proximity.

The German investigators in their survey of the means of infection do not give a high place to the fly. They consider that the soiled fingers of the carrier are more important. The Members of the Enquiry in India examined some 500 flies by plating methods, the flies having been captured in the neighbourhood of the latrines, and they were unable to find any suspicious colonies at all.

It has also been suggested that flies bred out of fæcal material may actually be hatched out with infected material in the intestine. Lumps of soil from recently made latrine trenches were taken to the laboratory and numerous fly larvæ removed, ground up and plated out, but no pathogenic bacteria could be found; nor could any pathogenic bacteria be isolated from the flies which hatched out. Also flies were caused to oviposit on infected fæces in the laboratory, but for days before the young flies had emerged the specific bacteria had already disappeared from the fæces and the intestinal contents of the flies bred out were found to be sterile, no growth at all resulting.

It is essential, however, that the fæces of infected persons should be disinfected either by boiling or by incineration or by chemicals and should never be exposed to the attention of flies, and where a water-carriage system of disposal of sewage is in existence this can be easily attained.

Prevention in General.

As the ultimate cause of enteric fever in general is specifically infected excreta, prevention consists in the first place in the detection and isolation at the earliest opportunity of every case of enteric fever and careful disinfection of their excreta, bed linen, &c. A known case of enteric fever should not be a danger in a properly equipped hospital, and, indeed, cases

of enteric fever are often treated in the general wards of a hospital, but rigid rules are necessary as to the disinfection of the hands of all concerned in nursing the cases.

One of the greatest dangers in the spread of enteric is the mild, undiagnosed ambulatory case, and here careful examination of all sick persons is necessary for diagnosis. The carrier is responsible for the endemicity of enteric fever, and as long as carriers exist in a population cases are liable to occur, possibly only at considerable intervals of time. Here, again, the known carrier who is of ordinary intelligence and capable of following out a few simple instructions regarding the disposal of his excreta and general cleanliness, and especially the cleansing of hands after defæcation, is not a great danger, provided that he or she is not in any way concerned with the preparation of food and especially the handling of milk.

General measures as to the water supply and sewage disposal have had a marked effect in reducing the incidence of enteric fever, and it is, indeed, only in primitive conditions in rural areas, or on active service, that the carrier is such a dangerous person. A sound axiom under such circumstances is to consider *all* excreta potentially infective and all water supplies potentially infected, and to take measures accordingly.

The usual method employed for the purification of water in the field is by means of chlorine obtained from bleaching powder, which must contain not less than 25 per cent. available chlorine. The method can be used with any container of suitable size, and is largely used in America for the treatment of water supplies of large towns. The water is first mixed with alum clarifying powder and then passed through canvas strainers into another tank, where the correct dose of bleaching powder is added. *B. typhosus* is killed in less than half an hour. The appropriate dose is ascertained by means of the Horrocks' water-testing box.

A new method of purifying water by means of chloramine is at present under trial and has already given satisfactory results. It has the great advantage that the water has no unpleasant taste after purification, and its germicidal effect on *B. typhosus* is at least as good as that of chlorine.

Detection of Carriers.

The ultimate decision whether a person is a carrier or not must rest on the isolation of the bacillus from the fæces or urine, or from the pus from a bone abscess. A case is on record where several men were infected from the discharge from an abscess of the ulna in a man of dirty habits.

Before commencing the examination of excreta it is necessary to narrow down the search as much as possible. In view of the well-known intermittency of the carrier condition, the examination of a large number of people is not only laborious, but may be quite futile ; whereas if we can narrow down the issue to a few persons, then frequent examinations can be made with some hope of success. The suspected persons may be selected on epidemiological grounds : for instance, if several families yield cases

at intervals, and it is found that the same cook had been employed in each family at the time the cases arose, then the matter is simplified ; or, again, if milk is suspected as a vehicle and cases have been traced to several farms at which one hand has been found to have been employed at the critical period.

The Widal reaction provides another method of selection. At one time it was thought that everyone who had passed through an attack of typhoid fever would show a positive Widal reaction for some years. It is now generally held that the majority of convalescents give a negative reaction six months after the fever, and the people who continue to give a positive reaction are very probably carriers. Thus, any person who gives a positive Widal reaction and who can in any way be connected with the outbreak should be carefully examined. This only applies in a non-inoculated population.

A skin reaction has been employed in the same way to discover a latent infection, and McKendrick (1923) has recently reviewed the work on this subject. Chantemesse (1907) employed as a diagnostic test a soluble toxin which he placed in the conjunctival sac ; this caused in patients or convalescents from enteric an acute inflammatory reaction which reached its height in 6 to 12 hours. Healthy persons, or persons with other diseases, did not so react. Austrian (1912) said that a toxin from numerous strains was necessary. Zupnik (1908) used the test by intracutaneous inoculation in a small series of cases and concluded that it was specific. Goodall (1908–9) investigated the ophthalmic reaction, using a solution of MacFadyean's typhoid endotoxin. He concluded that a positive reaction was in favour of a diagnosis of typhoid fever.

Floyd and Barker (1909) used the clear supernatant fluid from saline emulsions of virulent typhoid bacilli, applied as in Von Pirquet's test, in 36 cases of enteric ; 19 were positive, 9 negative. Gay and Force (1914) used a 5-days old glycerin broth culture of *B. typhosus* concentrated by evaporation at a temperature of 56° C. for 8 hours. They applied this to an abraded surface of the arm : 21 cases of typhoid were all positive except 1, and of 39 controls 35 were negative and 4 were positive. The test was also positive in inoculated persons. Later they used a dried preparation obtained from ' typhoidin ' by evaporation over sulphuric acid. 0·005 mgm. of this in 0·05 c.cm. was used as an intradermal injection. Thompson (1921) applied three loopfuls of T.A.B. vaccine to a scarified area, and suggested the use of this method for the detection of carriers.

McKendrick used an intradermal injection of Oxford agglutinable emulsions, which are formalinized. The dose was 0·04 c.cm. in children, and 0·08 c.cm. in adults. He injected four doses T.A.B. and control at intervals of one inch. His criterion of results was : *Negative :* Within 6 hours the small bleb which invariably appears has lost its whiteness and become faintly pink ; there is no induration, and by the third day the pink area has disappeared. *Positive :* For the first 48 hours

there is no difference between the positive and negative. By the third day the site of the inoculation is a dusky-maroon colour which is deepest centrally; it is slightly indurated, but not tender, and the induration extends beyond the area of the original bleb. The appearance on the fourth day is most characteristic and entirely different from that when the test is negative. There is a swollen, indurated, plum-coloured papule, with an extensive areola. On the fifth day it has commenced to fade, and on the sixth day there remains a slight induration and discoloration only. Induration alone should not be accepted as positive.

McKendrick tested 15 cases of enteric fever, 5 chronic carriers, 19 inoculated persons, and 360 controls, mostly children. Of the 15 cases of enteric all were positive, but for *B. typhosus* only; all cases were negative for *B. paratyphosus* A and B. The reactions became negative in convalescence in all but one case, which relapsed. *The five chronic carriers all gave a positive reaction.* None of the inoculated persons gave a positive reaction. This is contrary to Gay's experience. Of the 360 controls all were negative except two.

This work is very promising and should certainly be given a trial when any search for carriers is being made.

Fæcal carriers. The focus of infection in temporary or chronic fæcal carriers is the gall-bladder. The organism multiples in this situation and its elimination, at intervals into the intestine, is followed by its intermittent appearance in the stools. It is of the utmost importance, therefore, before declaring an individual free from the carrier condition, to obtain at least 5 or 6 negative examinations at intervals of 4 or 5 days. Owing to the irregular appearance of the organism in the fæces one single negative examination is of little value.

In investigating this condition it is usual to give a preliminary saline purge of 2 to 4 drachms of magnesium sulphate. This salt is to be preferred to other forms of saline purgatives as its presence in the duodenum causes active contractions of the gall-bladder with the consequent free flow of bile into the small intestine. A specimen of the fæces should be examined as soon after it has been passed as possible. If there is any unavoidable delay in making the investigation the specimen should be preserved in the glycerin and saline mixture previously noted. The technique followed in the isolation of the organism is similar to that already described. It is advisable to adopt some enrichment method such as that employing brilliant-green and telluric-acid, in addition to direct plating.

In acute cases of the disease it is frequent to find that in positive stools there is a high percentage of colonies of the pathogenic organisms evident on the plates. In carriers, on the other hand, no such free elimination of the organism is usual and the preponderance of colonies may frequently be of the lactose-fermenting type, with only a small number of colonies of typhoid or paratyphoid bacilli. It is, therefore, even more essential in the investigation of carriers to examine carefully the whole surface of the inoculated plate in case a few suspicious colonies may be overlooked.

In many of these chronic intestinal carriers, the continued infectivity of the gall-bladder has resulted in the formation of gall-stones which eventually calls for surgical interference. In a number of such cases *B. typhosus* has been recovered from the centre of the stones removed at operation.

Urinary carriers. With this more dangerous type of carrier the site of the infection is the pelvis of the kidney. In many urinary carriers the elimination of the organism is so intense that the existence of bacilluria can be readily determined by naked eye examination of the urine. In other cases the degree of bacilluria is slight, but in all there is a marked intermittence in the appearance of the organism in the urine. A co-existing cystitis is not uncommon in urinary carriers of long standing: in such cases there is a definite pyuria.

Isolation of the organism can be effected from a specimen collected with sterile precautions, either by direct plating or by plating subsequent to one of the enrichment methods. Owing to the intermittent appearance of the organism in the urine it is essential that at least four or five examinations with negative results should be obtained before excluding the possibility of the carrier condition.

D. H.

Practical Diagnosis of Typhoid and Paratyphoid Infections.

By H. Marrian Perry and H. J. Bensted.

Isolation of the Organism from the Blood.

In the early phases of typhoid and paratyphoid fever the infection is of the nature of a septicæmia and the causative organism can be isolated from the blood in a high percentage of cases. Although this fact is well recognized it is by no means uncommon for blood culture to be delayed in pyrexias of doubtful origin until the possibility of the successful recovery of the bacillus has become remote. It, therefore, cannot be too strongly emphasized that a laboratory diagnosis can be effected most readily by early culture of the blood in persons suspected to be suffering from one of the enteric group of fevers. The value of early blood culture is illustrated by the fact that in a series of 1,035 cases of enterica positive blood culture was obtained from 534 patients (Perry, 1918). It must, however, be mentioned that a considerable proportion of these cases were not inoculated either with typhoid or paratyphoid vaccine.

The most favourable period is within the first few days of the onset of the fever. At this stage of the disease the organism can be isolated from the blood-stream in the majority of these infections. The chance of success diminishes rapidly after the fifth or sixth day, and negative results are usual during the later stages of the illness. Should, however, relapse occur the organism may be again recovered from the blood.

Of the various possible methods, culture in fresh ox bile is perhaps the one which will yield the most uniform and satisfactory results. When the bile tubes have been inoculated they should be incubated at 37° C. and

subcultures made every 24 hours on agar slopes for a period of 6 days. In the greater proportion of cases yielding positive blood cultures growth of the organism will be obtained by the end of 48 hours. In inoculated individuals, however, it may be delayed for as long as 5 or 6 days. Daily subculture of the bile is, therefore, advisable in case growth has been inhibited.

In certain circumstances it may be necessary to perform blood culture at a distance from the laboratory, and to transmit the specimen by post. The most convenient method in such cases is to forward the bile in sterile 25 c.cm. vaccine bottles provided with rubber caps. The blood can be introduced directly through the rubber cap into the bile. Subsequent incubation and subculture are carried out in the usual manner. This improvization is, of course, unnecessary if a Kiedal tube or one of its modifications is available.

Isolation of the Organism from the Stools.

The successful isolation of the bacilli from the stools is dependent, to large extent, on the efficiency of the technique employed, but there is little doubt that the type of the infection is also a factor of some importance in the recovery of the organism. In mild cases of typhoid or paratyphoid, such as are frequently met with in individuals well protected by inoculation, the elimination of the bacilli in the stools may be extremely transient. In severe cases, however, it is usually more persistent.

As a rule the organism can be recovered from the fæces within three or four days of the onset of the disease, and is eliminated fairly consistently throughout the illness. Its appearance in the stools, however, may be most irregular and it is not uncommon to find that the examination may yield alternating positive and negative results, or a series of negative examinations with an occasional positive finding. It is evident, therefore, that several specimens should be investigated, and it is advisable to space the examinations by intervals of three or four days.

The stools should be invariably submitted to the laboratory at the earliest possible moment after they have been passed, the delay of even a few hours in the investigation of the specimen reduces the probability of the successful recovery of the organism. This precaution is particularly necessary in warm climates and its neglect frequently results in a negative finding in a stool that originally contained large numbers of typhoid or paratyphoid bacilli. Unless the freshly passed specimen can be received in the laboratory it is advisable to adopt some method of preservation to prevent the overgrowth of the less resistant pathogenic bacilli by *B. coli* and other saprophytic organisms. The best procedure to follow when delay in the examination is unavoidable is that of Teague and Clurman (1916). A sample of the fæces is emulsified in double the volume of 30 per cent. glycerin in a 0·6 per cent. solution of sodium chloride. This method of treating the specimens has given very satisfactory results when examination of fresh stools has not been possible.

The general technique of isolation and methods of cultivation are described in Vol. IX, and we need only mention here some special procedures that are useful for these purposes.

Enrichment methods. It has been recognized for many years that some dyes of the diamino-triphenyl-methane series (China-green, malachite-green, brilliant-green, &c.) when present in certain concentration in a culture medium exercise a selective action on the growth of typhoid-paratyphoid organisms, whilst inhibiting the development of the coliform bacilli. In the original application of this observation the dye was incorporated in a solid medium, but it was not until it was realized that the use of these dyes were of little practical value except in a fluid medium that their employment in the isolation of the enteric group of organisms became general. Of the dyes mentioned, brilliant-green has found most favour. It was later determined that the addition of telluric-acid to this dye enhanced its selective action on the growth of these organisms, more especially in the case of *B. paratyphosus* A and *B. paratyphosus* B (Bonney and Browning, 1918). Details of the method of use of these dyes are described later.

Emulsification of the fæces with petroleum ether has been recommended as a method of reducing the number of *B. coli* or *B. proteus* before culture of the stools. A small portion of the specimen is added to half its volume of ether and the mixture thoroughly shaken in a mechanical shaker for half an hour. A few drops of liquid from the bottom of the tube is removed with a sterile pipette and is plated in the usual manner (Bierast, 1914).

Differential media. The underlying principle of the many differential media devised for the isolation of these organisms depends upon the fact that they do not ferment lactose, whereas the coliform bacilli break up this sugar with the production of acid and gas. In all of these media, therefore, lactose is incorporated with an indicator so that the organisms producing acid form colonies that can readily be differentiated by their colour from the colonies of the non-lactose-fermenting bacilli.

A large variety of media is available for choice, such as Conradi-Drigalski, McConkey, Endo and litmus-lactose agar, &c. All of them will yield satisfactory results in expert hands, but probably that of Conradi-Drigalski is the most generally efficient. The preparation of this medium is, however, not without difficulty. Litmus-lactose agar is also an excellent medium and possesses the added advantage that it is easily made. One of the most important points in the preparation of any of the differential media, and more especially of litmus-lactose agar, is that overheating should be carefully avoided during any stage of their manufacture. Reference must here be made to the bismuth-sulphite medium of Wilson and Blair (1927). On occasions it yields very satisfactory results, but the difficulty of preparing different batches of exactly uniform quality tends to limit its use.

It will be more satisfactory for the worker to choose one or other of the above media, and to accustom himself to its method of preparation and to its employment, than to use a variety of media at different times.

Method recommended. A small portion of the freshly passed stool is emulsified in peptone water and, time having been given for the subsidence of the coarser particles, a drop is plated out on one of the differential media, preferably litmus-lactose agar. At the same time a tube of brilliant-green and telluric-acid broth (brilliant-green 1/200,000 and telluric-acid 1/25,000) is inoculated with one loopfull of the emulsion of fæces. The plates and tubes are incubated at 37° C. for 24 hours. At the end of this period the plates are examined, and if colonies of non-lactose-fermenting organisms are evident, the further investigation of the enrichment tubes need not be continued. If, however, non-lactose-fermenting colonies fail to develop on the plates a drop from the enrichment tubes should be distributed on a fresh plate of the medium, which is then incubated for 24 hours at 37° C. A negative result should be confirmed by a second plating from the enrichment tubes after a further 24-hours incubation.

Isolation of the Organism from the Urine.

The remarks that have been made regarding the detection of typhoid or paratyphoid organisms in the stools apply equally to the isolation of these bacilli from the urine. It must, however, be borne in mind that the elimination of the bacteria in the urine is more intermittent and of a shorter duration. In view of this fact the urine should be cultured at least three or four times during the course of the disease, as it is possible that if one single examination is made a transient bacilluria may be missed.

Method recommended. As a routine it is recommended that daily culture of the urine should be undertaken for 7 or 8 days during the second and third week of the disease. It is also advisable to employ some enrichment method such as culture in brilliant-green and telluric-acid broth (brilliant-green 1/150,000 and telluric-acid 1/15,000). The urine should be added in the proportion of one part of urine to two parts of broth, and the tubes should be plated after 24-hours incubation at 37° C.

The investigation of blood, stools or urine has now been carried to a stage where separate colonies have been obtained either on agar or on one of the differential media. The further steps necessary to confirm the identity of the organism that has been isolated are (*a*) the determination of its biochemical reactions, and (*b*) its agglutinability with specific sera.

Determination of Biochemical Reactions.

Although a large range of carbohydrate media can be employed in investigating the fermentative activity of these organisms, as a routine sufficient information can be derived from the use of lactose, glucose, mannitol and dulcitol. The tubes are inoculated with the organism under investigation either from an agar slope culture or an isolated colony on the differential medium, and are incubated at 37° C. The fermentation reactions

are noted at the end of 24, 48 and 72 hours. The typical biochemical reactions obtained with these carbohydrate media have already been mentioned (p. 24).

It should be noted that whereas the reactions of *B. typhosus* and *B. paratyphosus* B are well defined in 24 to 48 hours, those of *B. paratyphosus* A, particularly in dulcitol, may be delayed, and its typical biochemical reactions may only become evident on prolonged incubation. It is also to be noted that certain strains of *B. paratyphosus* A isolated in the Middle and Far East produce anomalous reactions that have caused confusion. The production of acid only in glucose and mannitol and no action on dulcitol are not uncommon. The true character of these aberrant strains can be recognized by serological tests.

The absence of indole formation by typhoid-paratyphoid bacilli is a further important characteristic in the identification of these organisms by biochemical tests. This fact should be determined by growth of the organism in peptone water. The culture should be incubated at 37° C., and tested at varying periods for three days.

It must be emphasized in connection with these biochemical tests that a recently isolated organism may vary, both in the rapidity of acid production and the degree of gas formation, from the same species that has been cultured for several generations. In the event, therefore, of a strain yielding ambiguous reactions it may be necessary to re-investigate its fermentative activity after a few subcultures.

The biochemical reactions of the organism isolated having been determined, its serological identification with specific sera is the next step in the investigation.

Serological Identification of the Organism.

The general principles of the agglutination test and the methods of carrying it out are discussed in Vols. VI and IX.

The technique of agglutination elaborated by Dreyer is largely used in this country and embodies the use of formalinized broth cultures and dilution of the serum by the drop method, as described by Donald (1913, 1915, 1916). It is only necessary to mention here that the suspension is prepared from a 24-hour broth culture of the organism under investigation. To this broth emulsion 0·1 per cent. of formalin is added, and it is diluted with normal saline solution until the opacity is equivalent to 200 to 300 million organisms per c.cm.

With the exception of certain strains, to which reference is made below, the serological confirmation by the agglutination test of *B. typhosus* and *B. paratyphosus* A usually presents no difficulty. It is necessary only to determine that the suspension of one or other of these organisms is agglutinated to approximately the titre of their respective antisera. In the case of *B. paratyphosus* B and *B. paratyphosus* C, however, complexity is introduced by the fact that the serum prepared against these organisms also agglutinates the allied members of the Salmonella group of bacilli.

In this connection confusion is most likely to arise between these strains of paratyphoid bacilli and the various types of *B. aertrycke* (Perry and Tidy, 1918). Differentiation can, however, be effected by the absorption of agglutinin test, the technique of which is described elsewhere. Another method of overcoming the difficulty is by use of mono-specific sera (Andrewes, 1922; Bensted, 1925) from which the heterologous agglutinins have been previously removed by absorption.

Certain fallacies must be borne in mind when investigating by means of the agglutination test the identity of any of these organisms.

Inagglutinability. It is not uncommon to find, when working with recently isolated strains, that the first subculture after isolation may fail to be agglutinated, or may be agglutinated to such a low titre, by the specific serum that doubt arises as to the interpretation of the result of the test. If this inagglutinability is due to recent isolation this fact will become evident after a few subcultures in broth, when it will frequently be found that the organism agglutinates to titre. In exceptional cases it may be necessary to continue the subcultivation for a prolonged period, as inagglutinability may persist through many generations.

In spite of the most prolonged subcultivation, however, certain of these strains never agglutinate to the titre of the serum (McIntosh and McQueen, 1914). In these rare cases advantage can be taken of the fact that an inagglutinable strain may retain its agglutinogenic property and be capable of producing a specific agglutinating serum on animal inoculation. A rabbit, should, therefore, be immunized in the usual way with the inagglutinable strain under investigation, and the agglutinative capacity of the serum subsequently tested against a normal type of the organism. An alternative method may be employed, using an immune serum prepared from a pure heat-stable antigen. It has been shown by Felix (1924) and others that the inagglutinability may be due to the absence or damage of heat-labile receptors in the particular strain during its existence in the body of the patient.

Spontaneous agglutination. If the fact is not recognized that, apart altogether from any specific action of the serum, the suspensions made from certain strains may clump spontaneously, error in interpretation will result. This phenomenon of spontaneous agglutination is more likely to occur with organisms that have been subcultured for some prolonged period, but may also happen in the case of recently isolated strains. It is more frequently observed when the saline suspensions are employed, it may, however, be also noted in broth emulsions. When this spontaneous clumping occurs it will be noted that a similar grade of agglutination can be seen in tubes containing different serum dilutions, and this fact will afford indication that the clumping is not due to any specific property of the serum. Any possibility of error can be avoided by including in the test a control tube containing the bacterial suspension and saline alone. The tendency towards the phenomenon can be diminished, or entirely obviated, by reducing the salt content of the suspension.

Co-agglutination. Reference has already been made to the fact that the interpretation of the result of the test may be rendered difficult owing to the presence of group agglutinins in the serum. It has been mentioned that this co-agglutination will occasion trouble only in the case of the Salmonella organisms. The employment of formalinized broth cultures has been found to minimize this group phenomenon to a considerable extent.

Group agglutination will be suggested if the organism under examination is agglutinated to only a fraction of the titre of the serum, the possibility of an insensitive strain having been excluded. A knowledge, therefore, of the ' end-point ' of the serum, with the particular technique of agglutination that has been adopted, is essential. The necessity for the application of the absorption test or of the employment of mono-specific sera has been already referred to.

Para-agglutination. This term is applied to the agglutination by a specific serum of an organism that bears no antigenic relationship to it. Thus, it may be occasionally observed that the non-pathogenic *B. coli*, isolated from the stool of a patient suffering from enteric fever, may show some degree of agglutination when tested against one or other of the specific sera. When rapid methods of agglutination, such as the slide method, are adopted in the investigation of colonies from a differential plate, the phenomenon assumes some importance, as mistakes may arise. The fallacy can, of course, be excluded by determining the biochemical reactions of the organism, but it is mentioned here to emphasize the importance of a complete cultural investigation of a bacillus before its final identification by serological tests. The property of para-agglutination is purely temporary and disappears on subculture.

Estimation of the Agglutinin Content of the Serum (Widal Reaction).

Although there are several methods sufficiently reliable for confirming the identity of a bacillus isolated from blood, stools, &c., it is obvious that for the purpose of research, or for repeated comparative estimations of the agglutinin content of a serum, a more accurate quantitative method is desirable. The technique devised by Dreyer is to be recommended, as it is the most accurate and sensitive method of carrying out the test available at present, and by it the variable factors that enter into the reaction are controlled. Dreyer's technique is fully described elsewhere (Vol. IX), so we need here only discuss briefly some of the main features which distinguish it from the other methods of agglutination.

The agglutinable emulsion. The variability in sensitivity to agglutinaton of emulsions prepared from the same strain of organism by different methods is one of the most important factors to control in standardizing the test. It can readily be determined that an emulsion prepared from a strain of bacillus that has been cultured for a period on solid media is less agglutinable than an emulsion of the same strain that has been cultivated in broth. This variation is often so evident that there may be

little comparison between the end-point of the serum when it is tested with a broth and with a saline suspension. The opacity of the suspensions has also an important relation to the delicacy of the test. Thick saline or broth suspensions yield a lower end-point and a more gross type of agglutination.

The outstanding feature of Dreyer's technique is the recognition of the importance of standardizing the method of preparation of the bacterial emulsion and the determination of the sensitivity to agglutination of emulsions made at different times.

Many workers have hesitated to accept the possibility of standardizing the agglutinability of a bacterial suspension on the grounds that this property is so unstable that an emulsion may vary in its agglutinability on storage. Whilst this contention may be well founded in the case of recently prepared bacterial emulsions, Dreyer and his co-workers (1905 and 1909) have published evidence that the agglutinability of formalinized broth cultures prepared in the above manner becomes stabilized after a few months storage. They have further demonstrated that the coefficient of agglutinability shows little or no change over a period of years. It is thus possible to give to different batches of emulsions factors illustrating their sensitivity to agglutination and to express the results of the test in standard agglutinin units.

Qualitative receptor analysis. The work of Felix (1924), Burnet (1924) and of Arkwright (1927), which calls attention to the heat-stable, small-flaking agglutinins which usually appear in the serum of patients suffering from enteric fever, has been discussed on pp. 27–29. This observation would appear to be of definite value in distinguishing between pure inoculation agglutinins and those due to inoculation plus a natural infection. Felix has produced evidence to show that, if, in the serum of a patient, small-flake agglutinins can be distinguished mixed with large-flake agglutinins, a diagnosis of enterica can be made. It is usually not possible in these cases to distinguish between infection due to one or other of the three serological types, owing to the fact that group agglutination between typhoid and paratyphoid A and B is caused by the small-flake agglutinins.

The technique of Felix, which differs essentially from that of Dreyer, should be followed closely and even then considerable experience is necessary in the interpretation of the results.

Inhibition zone phenomenon. Attention must here be directed to errors in interpretation due to the occurrence of what is known as the inhibition zone phenomenon. In some high-titre sera it is not uncommon to observe that, whilst no agglutination occurs in the lower dilutions, clumping of the bacterial emulsion becomes evident as the dilution of the serum increases. When this zone phenomenon occurs it does not usually extend beyond dilutions higher than 1/50 or 1/100, although it may occasionally be noted in dilutions as high as 1/500. It is only in the event of the inhibition zone extending beyond the range of the dilutions put up that a mistake is likely to arise. By reading the results for a second time

after a period of 24 hours at room temperature, the occurrence of the phenomenon is unlikely to lead to error, as at the end of this period the higher dilutions usually show some evidence of agglutination, and the true end-point of the serum can then be determined by putting up a second test that covers a still higher range of dilutions. The actual cause of the phenomenon is not clear.

PRACTICAL APPLICATION OF THE AGGLUTINATION TEST IN DIAGNOSIS.

The application of the agglutination test yields information of the greatest value in the detection of typhoid and paratyphoid infections, but it should be subsidiary to diagnosis by hæmoculture or by recovery of the organism from the stools or urine.

The value of the test requires separate consideration in the case of non-inoculated individuals and those who have previously received prophylactic typhoid-paratyphoid vaccine.

Value of the Test in Non-inoculated Individuals.

It is in this class of patient that the agglutination method of diagnosis assumes its greatest value. Normally the serum of individuals who have not previously suffered from typhoid or paratyphoid fever, or who have not been inoculated against these diseases, does not, even in low dilution, agglutinate the organisms. If, however, a history of previous infection or inoculation is elicited from the patient, it must be borne in mind that residual agglutinins may persist in the serum for a period of years, and that it may agglutinate one or other of the bacilli in a dilution that would be considered diagnostic in the non-inoculated. In the absence, however, of a previous history of inoculation or infection, the presence of agglutinins in the serum may be accepted as evidence of active disease, provided that certain fallacies are taken into consideration.

Period of the disease. The period of the disease at which the test is made has an important relation to the interpretation of the result. Agglutinin production is not usually evident before the sixth or seventh day. A negative result obtained in the earlier stages of the illness should, therefore, be confirmed by a further test during the second week of the fever. This lag in agglutinin response may occasionally be delayed until the tenth or eleventh day, whilst in very severe and toxic cases the response may be entirely absent.

The maximum degree of agglutinin production is observed from the eighteenth to the twentieth day of the disease. From this onwards the titre of the serum declines uniformily and rapidly to a level where it remains almost unchanged for a period of years.

Normal agglutinins. The fact that the serum of some normal individuals, from whom a previous history of infection cannot be obtained, agglutinates emulsions of the organisms in low dilution may cause error in diagnosis. Fortunately the presence of ' normal ' agglutinins for typhoid and paratyphoid bacilli is so infrequent that they are not an important

cause of error in diagnosis. Even when the phenomenon is observed agglutination occurs only in low dilution. Should, however, doubt arise that a positive result is due to the presence of these 'normal' agglutinins a second estimation demonstrating a rise or fall in the titre of the serum, dependent on the stage of the illness, will indicate active infection.

Degree of agglutinin production. As above noted, the degree of agglutinin production and, therefore, the resulting titre of the serum is related to the period of the illness at which the test is made, but it also depends upon the reaction of the patient to the infection. Attention has been drawn to the fact that in very severe infections there may be an entire lack of response, and in certain mild cases of infection agglutinin appearance may be so slight that the titre of the serum, even in the second week of the disease, may not be considered diagnostic. In such cases, however, repetition of the test later in the course of the illness may demonstrate that the agglutinin content has increased to such a degree that a diagnosis can be made with certainty.

In infections with *B. typhosus* or *B. paratyphosus* B the lowest diagnostic titre of the serum usually accepted as positive is a dilution of 1 50. During the second week of the infection it is the rule, however, to obtain positive reactions in very much higher dilutions. With *B. typhosus* a positive result in a dilution of 1,50,000 or even higher, is not infrequent, whilst with *B. paratyphosus* B agglutinin response may be still more pronounced and the serum may react in a dilution of 1/500,000. These high titres are in contrast with the results observed in infections due to *B. paratyphosus* A. The agglutinogenic property of this organism is less marked, and it is not uncommon to find in this disease that the serum may fail to agglutinate the bacillus in a dilution higher than 1 25. Although in mild infections a feeble agglutinin response is common, should relapse occur the agglutinative capacity of the serum shows a marked increase; the reaction is, however, rarely as marked as in the other two infections.

Value of the Test in Inoculated Individuals.

Agglutinin production following inoculation. Before assessing the value of the agglutination method of diagnosis in the case of inoculated individuals it is necessary to consider briefly the changes that occur in the agglutinative reaction of the serum subsequent to inoculation. The course of agglutinin production may be readily followed by making repeated estimations (usually at intervals of four or five days) of the agglutinin content of the serum after the injection of the usual dose of typhoid-paratyphoid vaccine. During the first four or five days following the inoculation there is a similar lag in response to that noted in the active infection. The precise period at which the serum reacts positively is subject to some slight variation, but about the sixth day demonstrable agglutinins appear. From the sixth day onwards the agglutination titre rises rapidly to its maximum, which is attained between the sixteenth and

twentieth day after inoculation. The peak of agglutinin production may, in some cases, be delayed until the twenty-fourth day. As a rule from the sixteenth to twentieth day the titre of the serum declines, at first rapidly and then more slowly, until about the eighth week after inoculation, when repeated estimations, at the intervals mentioned, demonstrate no measurable decrease. Inoculation agglutinins may subsequently persist in the serum for a period of some years to an extent that varies in different individuals, but for at least one year after inoculation the agglutination titre of the serum may be relatively high.

The effect of the usual double inoculation is that a higher degree of agglutinin response is manifested and that inoculation agglutinins persist for a longer period than is noted after a single injection.

Inoculation of ' Mixed Typhoid-paratyphoid Vaccine ' is followed by precisely similar results, except that agglutinins for each organism are produced independently. The serum shows the same rapid rise in titre followed by the decline to a level at which it remains comparatively stable.

Different individuals show considerable variation in agglutinin production, but the response following the inoculation of *B. typhosus* and *B. paratyphosus* B is usually well marked and the serum maintains a comparatively high titre for some years. In the case of *B. paratyphosus* A, however, this response is rarely evident to the same extent.

Fluctuations caused by active infection. From the foregoing it will be appreciated that in the case of inoculated individuals no deduction can be made from one single estimation of the agglutinin content of the serum. Such cases almost invariably show some degree of agglutination for all three organisms, due to the residual inoculation agglutinins, and very recently inoculated persons may show comparatively high titres. Before attempting a serological diagnosis of active typhoid or paratyphoid infection it is essential that three or more successive examinations should be made at intervals of three or four days to determine if changes in agglutinin titre occur, such as are known to accompany the active disease. As the validity of the deductions made from such tests is dependent on the accuracy of comparative observations, the necessity for a standard technique employing suspensions of known sensitivity to agglutination is obvious.

If, then, a series of agglutination tests are made in the case of an inoculated individual who has contracted typhoid or paratyphoid fever very similar changes to those following inoculation will be noted. There is the same lag in the production of agglutinins for the infecting bacillus, followed by a sharp rise and subsequent decline, the only difference being that the level to which the titre may rise is more variable and the decline more rapid than is observed after inoculation. In such cases, however, the rise commences from the level of the residual inoculation agglutinins and declines towards the higher base line.

Coincidently with, or slightly antecedent to, this rise and fall, certain changes may be noted in the agglutination titres for the other two

organisms. Firstly, no measurable change may be observed. Secondly, a slight rise in the titres may be noted, which is followed by a decline towards the original level. Thirdly, a sharp rise may occur at the same time as the increase in agglutinins for the infecting organism, followed by the usual fall to the former level. In some instances the changes in titre for all three organisms occur synchronously and equally, when it may not be possible to identify the exact type of infection. The rise in titre for the infecting organism is, however, usually so markedly accentuated that there is little difficulty in determining the member of the group responsible.

Presuming then that a standard technique, such as that of Dreyer, is employed, a change in titre for one organism of not less than 200 per cent., manifesting itself as a rise and fall at the appropriate period of the illness is usually accepted as evidence of active infection. Exception must, however, be made in case of infection due to *B. paratyphosus* A, as the agglutinin response towards this organism may be so little marked as to render diagnosis uncertain or impossible. When repeated examination of the serum yields results that are almost level or that exhibit less marked variations than the above, the tests may be interpreted as negative.

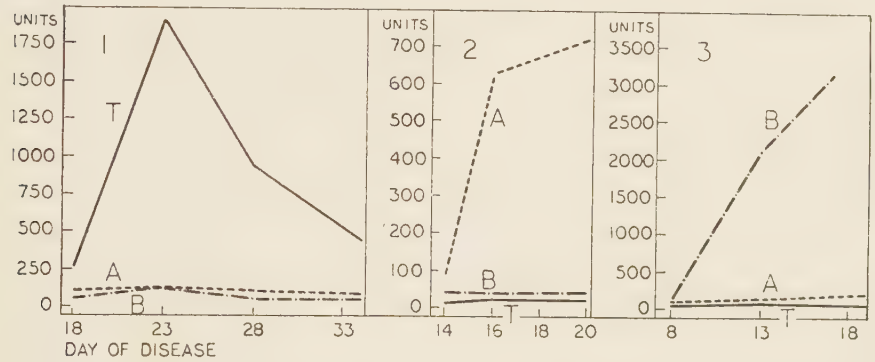
It must be remarked that the agglutination method of diagnosis in ' triple ' inoculated individuals has been the subject of much controversy. Criticism has centered chiefly round the assumption that during the course of various febrile conditions other than enteric fever, the inoculation agglutinins may exhibit changes that could be regarded as diagnostic of active typhoid or paratyphoid infection. For example, it has been stated that marked changes in titre have been demonstrated in miliary tuberculosis, pneumonia, septic infections, typhus, trench fever, &c. (Felix, 1924).

In many instances it is evident that the necessity for the employment of a standard method of making these comparative estimations has not been appreciated, and that such changes in the agglutinin titre, as have been observed, could be attributed to experimental error (Topley, Platts and Imrie, 1920). Further, workers who have had a wide experience of Dreyer's technique have been unable, in conditions other than enteric, to detect differences that would cause errors in diagnosis (Walker, 1916 ; Perry, 1918).

This agglutination method of diagnosis should be applied as early in the course of illness as possible. An estimation made before the sixth day of the disease, that is at a period before agglutinin production becomes evident, is of value in determining the level of the inoculation agglutinins. Repetition of the test will then illustrate any change in the titres that may occur and a rapid rise in the agglutinin content of the serum for one of the organisms will enable an early diagnosis to be made. Should, however, the case come under observation at a later period of the illness it is possible that the maximum rise in titre may take place in the interval between the two observations.

In such cases it is only by plotting the graph of the whole series of tests made that any deductions are possible. Cases investigated after

the twenty-fourth day of the disease show a rapid and regular decline in agglutinins. Should relapse occur during this period, the titre of the serum will again rise rapidly and may exceed the level previously attained. As a routine measure the serum of cases coming under observation for the first time should be tested in a series of dilutions of 1/50 to 1/500. If no agglutination is evident in this series or if the ' end-point ' is not reached the test should be repeated with the serum appropriately diluted. The practical application of this method of diagnosis is best illustrated by reproducing graphs representing the course of agglutinin production in inoculated individuals from whom the infecting bacillus had been recovered from the blood, fæces or urine.



Graphs of agglutinins in serum in (1) a case of typhoid fever, inoculated with T.A.B. vaccine, Sept., 1916, admitted to hospital with fever, Nov., 1916; (2) a case of paratyphoid A fever, inoculated with T.A.B. vaccine, June, 1916, admitted to hospital with fever, May, 1917; (3) a case of paratyphoid B fever, inoculated with T.A.B. vaccine, June, 1916, admitted to hospital with fever, April, 1917. (After H. M. Perry.)

The method of 'qualitative receptor analysis' introduced by Felix, to which reference has already been made, may usefully be employed to determine the presence of active enteric group infection in inoculated individuals.

Post-Mortem Diagnosis.

Under favourable conditions the organism can be isolated at autopsy in a large number of cases. The interval that has elapsed between the death of the patient and the post-mortem examination is an important factor in the success of the investigation, more especially in warm climates. In tropical countries, a delay of more than a few hours may render isolation impossible, as secondary invasion of the blood and organs with *B. coli* and allied organisms occurs soon after death.

Isolation of the organism may be attempted from the blood, spleen, gall-bladder, intestinal lymphoid tissue, including mesenteric glands, and the fæces. At whatever stage of the disease death has occurred the

bacillus can be most readily recovered from the spleen. When this organ has been exposed, its capsule is sterilized by searing an area with a red-hot searing iron. A small amount of splenic pulp and blood are collected by plunging a sterile sharp-pointed pipette, fitted with a teat, through the seared area into the interior of the organ.

A specimen of bile can, at the same time and in the same manner, be obtained by puncture of the gall-bladder. In certain cases, where the bile is particularly viscid or the wall of the gall-bladder fibrosed and thickened by old inflammation, the bile may be more easily collected by making an incision in the wall.

H. M. P. and H. J. B.

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CHAPTER II. THE SALMONELLA GROUP.

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Definition.

A large genus of serologically related, Gram-negative and non-sporing. bacilli ; $0 \cdot 4$ to $0 \cdot 6\mu \times 1$ to 3μ in usual dimensions, but occasionally forming short filaments ; showing, with certain exceptions, a motile peritrichous phase in which they normally occur : in fact, adhering to the pattern of *B. typhosus* in staining properties and morphology. Failing to ferment lactose or saccharose, to clot milk, to liquefy gelatin or to produce indole, they regularly attack glucose, usually with, but occasionally without, gas production. All the known species are pathogenic for man, animals, or both.

Classification.

Present Conclusions with Regard to the Subdivision of the Salmonella Group : Nomenclature.

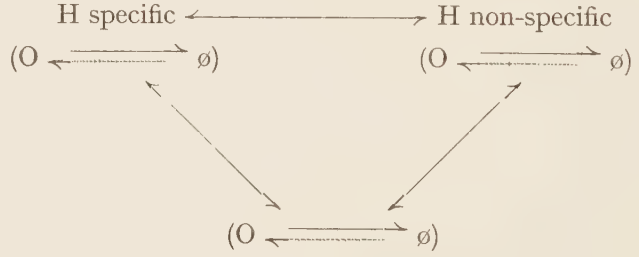
During recent years, with advent of exact and analytic methods of serological comparison, it has become evident that, in addition to the limited series of commonly occurring forms recognized by the pioneers of the group, there occur others of local or temporary importance and a larger—perhaps very large—number of rare types which come to light from time to time. Using Schütze's (1920) types as primary basis for study, the writer (White, 1925, 1926) has endeavoured to effect some unification of Salmonella taxonomy by comparison of such representative cultures as have been available : making use of comparative studies by others, his main conclusions are set out *seriatim* below. The *Schottmüller type* of Schütze coincides with the ' Echter Paratyphus B ' or *B. paratyphi B hominis* of the German Kiel School, with the Group IV of Hecht-Johansen, and with the *B. paratyphosus B* of British writers ; for the rest it has been, in many cases, but one of the forms embraced by the term *B. paratyphi* (*-us, -osus B*) ; for present purposes *B. paratyphosus B* may be accepted as type name. The *Mutton* type of Schütze has been identified with the ' Hatton ' strain of Durham (1898) and the ' Aertryck ' strain of de Nobele (1899), with representative Breslau type strains sent by various German workers, with Hecht-Johansen's Group VII, and with the Freiburg type of Uhlenhuth (1925) ; it includes the majority of so-called ' animal paratyphoid ' strains, *B. typhi murium* of Loeffler—but not all races so named—*B. psittacosis* (Nocard), the ' swine typhus ' and ' calf typhus ' strains of TenBroeck (1918, 1920), *B. pestis caviæ* of Wherry (1908) and

other American authors, and at least some of the strains termed *B. anatum ;* it has been the main contaminant of the *B. paratyphus* (*-osus*) *B* of Continental and American authors : in this article the name *B. aertryck* (Aertryck type) is adopted as being firmly established in this country ; continued use of the term *B. suipestifer* for races of this type is to be condemned. Schütze's *Binns type* must be regarded as a loss variant of *B. aertryck* and corresponds with the Group VI of Hecht-Johansen and probably with the ' Breslau ' type of Uhlenhuth (1925). The *Reading* and *Newport* types of Schütze have escaped recognition in other systems ; the ' Paratyphus β_2 ' strain of Weil and Saxl (1917) is of the latter type. The peculiarities of the *Stanley* type have probably been several times described ; closely allied to, but distinct from this organism, is the *Bombay* type of Basu. The ' *G* ' *Arkansas* and *Hirschfeld* types of Schütze present a classificatory problem of their own (see White, 1926). It is convenient to speak of a *Suipestifer-Hirschfeld* cluster of types comprising : (*a*) an *American type* (Hog-*cholera b., B. choleræ suis, B. suis, B. suipestifer* of American authors = Arkansas type of Schütze = Group I Suipestifer of Andrewes and Neave, 1921 = Hog cholera b. of Aoki) ; (*b*) an *Eastern type* (*B. paratyphosus C* of Hirschfeld, 1919 = Hirschfeld type of Schütze = Erzindjan type of Neukirch, 1917 = *B. paratyphus* β_5 of Weil, 1917 = Paratyphus N_1 bacilli of Russian authors = Paratyphus C_2 b. of Weigmann, 1925) ; (*c*) an *European type* (G type of Schütze = Group II Suipestifer of Andrewes and Neave = Group V of Hecht-Johansen = Paratyphus β_1 of Weil and Saxl, 1917 = Kunzendorf type of German authors = *B. paratyphosus C* of Heimann, 1912 = *B. paratyphosus C* of Dudgeon and Urquhart, 1920, and Wordley, 1923 = ' *B. voldagsen* ' of Bernhardt, 1913) ; (*d*) a *Glässer-Voldagsen* type (= Ferkel typhus b.). To the types so far enumerated must be added *B. abortus equi* (*abortivo equinus, paratyphi equi*) and a number of forms not included in Schütze's original series. *B. paratyphosus A* (*B. paratyphi* of Bergey) is brought into serological continuity with the main body of Salmonella types by the mediary of the ' atypical paratyphus A bacillus ' of Aoki and Sakai (1925)—a form for which we adopt the designation *Sendai type.* In the case of *B. enteritidis*, Gärtner (Enteritidis or Gärtner type) similar continuity is effected through the *Tokyo* type (N strains of Sakai, 1925) and in close serological affinity stand the *Dublin type* of Biggar and White (= *B. enteritidis* of Pesch, 1926) and the *Derby type* (Peckham, 1923 ; White, 1925). *B. typhosus* (Typhosus type) is from the serological, as from the clinical, point of view a typical Salmonella and must be classified as such ; so too *B. sanguinarium* (*B. gallinarum* Klein = *B. typhi gallinarum alkalifaciens* Pfeiler and Rehse = fowl typhoid or Hühnertyphus bacillus) and *B. pullorum* Rettger. To these must be added a few rare forms—the *L type* of White (1926), the *Thompson type* of Scott (1926), the *Dar-es-Salaam type* of Schütze and *B. morbificans bovis* of Basenau (1893)—and two more important forms : to one of these, represented by the Paratyphus N_2 strains of Russian authors (= Paratyphus C_1 of Weigmann, 1925), the

name *Moscow type* may be assigned; the second is *B. abortus ovis* of Schermer and Ehrlich, 1921 (= *B. paratyphi ovis* of Lütje)—a form which the writer has had no opportunity to examine.

A Descriptive Classification of the Salmonella Types.

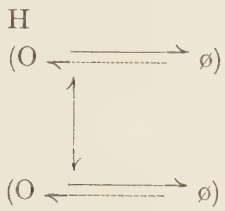
It is now clear that the older plan of formal division of the strains into Paratyphus A, Enteritidis, Paratyphus B and Suipestifer groups, constituting together a larger group or genus of the same standing as the typhoid group or genus *Eberthella*, is inadmissible; all these forms, *B. typhosus* included, are inextricably involved in one continuous system of antigenic affinities. In a loose way the terms may be used to refer to certain clusters of types showing specially close serological relationship, but this is a matter of descriptive expediency, not of strict classification. There is, however, another basis on which, for purposes of description, the genus *Salmonella* may be cleft almost cleanly into three groups: this by reference to the variation phenomena in the several types (White, 1926). At present three forms of antigenic variation are definitely known to occur within the group: (1) change, usually reversible, from a motile flagellate state, exhibiting both a very labile (H) and a very stable (O) antigen, to a non-motile aflagellate state in which the latter, the stable (O) antigen is present; this is the *H-form — O-form variation* of Weil and Felix; (2) change, sometimes reversible, perhaps, when complete, irreversible, of the stable (O) complex of the organism into a serologically different complex (ø) which is debased in specificity; this change, named with reference to the cultural modification with which it is associated, is the *smooth-form — rough-form variation* of Arkwright; (3) saltative oscillation of the labile (H) antigenic complex between two serologically contrasting states (H specific and H non-specific) characterized respectively by relative specificity and group non-specificity of behaviour; this is the *specific-phase — non-specific-phase variation* of Andrewes. On this understanding we may represent the variation system of the typical Salmonella by Scheme I. Salmonellas which exhibit this full range of modification



Scheme I, Diphasic Type.

may be termed *diphasic* in that they present the phase alternatives of Andrewes. Certain Salmonella types, however, show a lesser range of variation. A number which may be termed *monophasic* show the labile (H) antigenic complex in one form only, that form corresponding, it seems,

in some cases with the specific, in others with the non-specific phase of diphasic species ; such types are subject only to O-form — H-form and smooth-form — rough-form variation (Scheme II). A still simpler condition is seen in a few types which, seemingly devoid of labile antigen in appreciable amount, show only change from the normal smooth to the degenerate rough condition (Scheme III) ; these we will term *Meta-Salmonella types.*



Scheme II, Monophasic type.

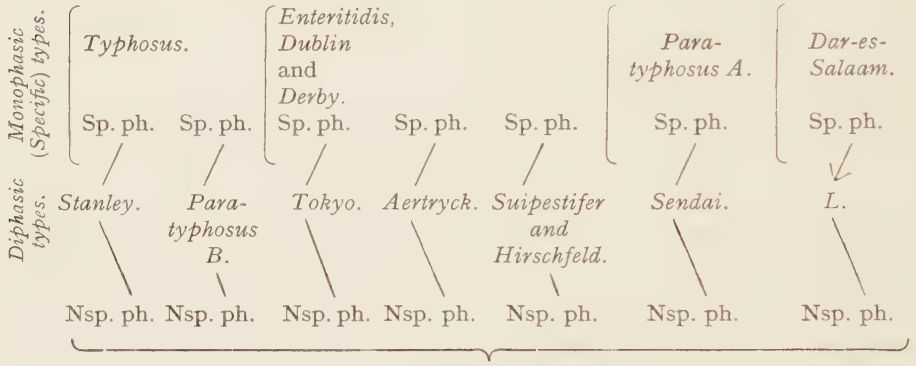
Scheme III, Meta-Salmonella type.

We have thus before us three sharply defined serological grades of Salmonella—diaphasic, monophasic and meta-Salmonella—among which the known types range themselves as follows : —*Diphasic Types :* Paratyphosus B, Stanley, Bombay, Aertryck (except the Binns Strains), Newport, Reading, Morbificans Bovis, Sendai, Tokyo, L, Thompson, Hirschfeld, American Suipestifer and Glässer's bacillus ; *Monophasic Types : 'specific'* : Typhosus, Paratyphosus A, Enteritidis, Dublin, Derby, Moscow, Abortus Equi, Dar-es-Salaam, and (probably) Abortus Ovis ; *'non-specific'* : European Suipestifer, Voldagsen bacillus and the Binns strains of the Aertryck type ; *Meta Salmonella Types :* Pullorum and Sanguinarium. This division of the group, useful as it is, must in no wise be regarded as a real classification.

Interrelationships of the Salmonella Types.

While, apart from defining the individual types, recent serological studies have failed to discover any natural basis for subdivision of the group, they allow some opinion to be formed as to the relationships of certain types ; the matter has been discussed by White (1926). Though, with a few exceptions, the types of the diphasic series are sharply marked off from one another by the serology of their specific phase races, they are welded in one agglutinative federacy by the generalized reactions of the corresponding non-specific phases. There can be no hesitation in accepting the diphasic series, which approximates to the old Paratyphus B group, as a natural group of species derived from a common evolutionary source. Among the monophasic types serological signs of affinity are very irregularly distributed : very striking, however, is the serological similarity of certain of these monophasic types to the *specific* phases of particular diphasic forms (Scheme IV) : *B. paratyphosus A* corresponds with the specific phase of the Sendai type both in labile and stable antigen ;

B. enteritidis holds an identical relation to the specific phase of the Tokyo type, with which the Enteritidis-like Dublin and Derby types also show marked similarity ; *B. typhosus* coincides in its labile complex with the corresponding specific complex of the Stanley type and exhibits a similar though lesser relation to that of the Bombay type ; in labile antigen the Dar-es-Salaam type overlaps extensively with the specific phase of the L type. In certain other cases monophasic forms have been found to coincide with the *non-specific* phase of diphasic forms : the Binns strains are practically identical with the non-specific races of *B. aertryck* and European *B. suipestifer* with those of the American Suipestifer type.



Brackets indicate range of major agglutinative interaction of the flagellar reagents.

Sp. ph. = Specific phase.

Nsp. ph. = Non-specific phase.

(A number of types are omitted from the scheme to avoid complication.)

SCHEME IV.

This remarkable phenomenon, the recurring relation of monophasic types—otherwise largely isolated in their serology—to the specific phases of individual diphasic types, or *alternatively* to the various non-specific phases, can no more be attributed to mere coincidence than can the periodicity of the chemical elements. The writer has ventured the explanation that the monophasic types are derivatives of diphasic Salmonellas extant or extinct, by suppression of one phase—the non-specific or the specific as the case may be ; in both cases the event must have occurred repeatedly, affecting now this, now that line of Salmonella descent and yielding a series of monophasic types of polyphyletic origin. The writer interprets *B. sanguinarium* and *B. pullorum*, the known representatives of the meta-Salmonella series, as permanently aflagellate (or ' O ') derivatives of an Enteritidis-like ancestry. In our opinion the types of the monophasic and meta-Salmonella series simply result from the stabilization of conditions which in the diphasic Salmonella are labile—that is to say, transmutable.

Serological Definition of the Genus.

Hitherto, whatever the basis adopted for subdivision of the Salmonella series, taxonomists have relied on biochemical criteria for delimitation of the group as a whole : the definitions presented, where comprehensive, enclose a genus of altogether doubtful reality ; where rigidly framed, are subject to violation and clash with the now known facts of serological relation. Consideration of the normal serology of the Salmonella types has disclosed an intricate system of serological affinities giving, so to speak, a statement of relationship by relays : it, however, assists but little in setting limits to the natural genus. The writer (White, 1929) has sought to develop a second basis for serological grouping depending on the observation of Schütze (p. 107) that rough variants of the Salmonella group show, with rough Salmonella antisera, a range of cross-agglutination out of all proportion to that of the corresponding smooth reagents. It seems that, while rough variants of the established Salmonella types show almost complete agglutinative community with regard to the essential elements of the rough state, they exhibit a marked degree of group specificity in the same respect. The observations allow definition of the genus as a group of serologically related forms, and do something to map out its confines, supporting inclusion of *B. typhosus* and exclusion of certain organisms, such as *B. columbensis* and *B. morgani*, admitted by various authors.

Conditions of Life and Behaviour in Culture.

Limiting and Optimum Hydrogen-ion Concentrations for Growth.

For A and B paratyphoid bacilli the limits appear to be about pH 4 to 4·5 on the acid side, and 8·5 to 9 on the alkaline, the optimum zone being approximately pH 6·5 to 7·5. It is generally agreed that *B. typhosus* is definitely more susceptible to the growth-inhibiting action of alkali than are the aerogenic organisms named, which are in turn rather more susceptible than most coliform bacteria.

Longevity and Viability under Favourable and Unfavourable Conditions.

On nutrient *agar* and in *broth* all Salmonella bacilli remain viable for many months, and, on the former medium at least, may survive for many years, provided the tubes are hermetically sealed to prevent loss of moisture and that the nutritive constituents of the medium are not so readily available as to be rapidly exhausted and to flood the culture at the outset with the harmful products of its growth. In fermentable *carbohydrate media*—unless the carbohydrate concentration be small (0·1 per cent.)—rising acidity limits the life of the Salmonella to a few days.

It is impossible to do more than exemplify scattered observations regarding survival outside the conditions of laboratory culture. The

experiments of Fletcher (1917–18) and Bumke (1925[1]) regarding the survival of Salmonella bacilli *in fæces* are of interest. The former found that in fæces exposed to evaporation on swabs paratyphoid bacilli remained viable for 10 days at 15° C., and 4 days at 37° C. ; in a saline emulsion of fæces survival of A and B paratyphoid bacilli was observed beyond 100 days ; on the whole the experiments indicated the greater hardiness of the B type. Through 155 days, Bumke isolated *B. paratyphosus B* from the buried fæces of a carrier ; from dried fæces paratyphoid bacilli are said to have been isolated after lapse of two years. *B. suipestifer* has been isolated from dung after 36 days, and from carcasses up to 160 days. The tolerance of the Salmonellas to desiccation is considerable. Heim (1905) found mouse typhoid bacilli, dried in mouse-blood on silken threads and kept over anhydrous $CaCl_2$, alive at the end of 22 months. There is a record of survival of *B. suipestifer* for 150 days under similar conditions. In *physiological saline*, *tap water* and *distilled water* Salmonella bacilli may persist for months though their number usually decreases rapidly during the first week or ten days ; it is alleged that *B. suipestifer* has been recovered from drinking-water after 18 months. In *carbonated water* according to Koser and Skinner (1921) typhoid and paratyphoid bacilli die out in a few days at room temperature ; less rapidly at 1° C. Russ (1916) found that paratyphoid B bacilli might survive on various *fruits* to between the fourteenth and thirty-first day, and on certain *vegetables* up to the twenty-fifth to thirty-eighth day.

Dold (1921) found that in a 1 to 4 per cent. extract of black *tea* typhoid and paratyphoid B bacilli lived for about 80 days ; in 6 per cent. extract of *coffee* the latter organism survived for about a fortnight, considerably outliving *B. typhosus*. In the liquors of most *cooked vegetables*, Salmonella bacilli multiply rapidly ; in *Sauerkraut* and *fruit juices* they die out rapidly (Koser, 1922). In *souring milk* they are sooner or later destroyed : according to Heinemann (1915) they are destroyed by 0·45 per cent. of lactic acid : Savage and White (1925[2]) were, however, able to demonstrate *B. aertryck* in cheese, inoculated in the making, up to the twenty-fourth day, though at the twenty-first day the acidity corresponded to 1·48 per cent. of lactic acid. Among studies regarding the disinfectant action of the *gastric juice* that of Scheer (1919) may be noted ; it was found that, like dysentery bacilli, typhoid and paratyphoid B bacilli were killed within two minutes ; their susceptibility was rather greater than that of *B. coli*.

With its bearing on the practice of pickling meat the resistance of Salmonella bacilli to *salt* is of interest. While, with a little variation with strain and type, a salt concentration above 7 to 8 per cent. brings Salmonella growth to a standstill, considerably higher concentrations fail to effect quick sterilization of cultures growing on agar, in broth or in meat. In Weichel's (1910) experiments agar cultures of *B. enteritidis*, *B. aertryck* and *B. paratyphosus B* covered with crystalline salt remained viable for two months ; at room temperature agar cultures covered with

15 per cent. salt solution and broth cultures containing 25 per cent. of salt showed viable bacilli up to the thirty-third day, and, where lower salt concentrations were employed, for much longer periods. When, however, inoculation was made into media already salted (12 to 15 per cent. of NaCl) and incubation carried out at 37° C. the bacilli died out more rapidly. At ice-box temperature destruction of the organisms was greatly retarded : even in 25 per cent. concentration salt failed to effect sterilization of grown broth cultures in three months at 0 to 4° C. In other experiments Zwick and Weichel (1910) found that salt concentrations up to 19 per cent. did not completely destroy food-poisoning organisms, in meat already infected, before the seventy-fifth day. These experiments indicated that the effect of salt varies not only with the concentration of the reagent, but also with the medium and temperature of its action and with the concentration of the bacilli on which it acts.

As regards the disinfectant action of other chemical agents, one is faced with a scattered literature in which discussion of the ærogenic Salmonellas is for the most part secondary to that of *B. typhosus ;* actually the gas-forming members of the group approximate closely to this last in toleration and susceptibility.

Resistance to heat. Statements as to the thermo-resistance of the common types have not been in entire harmony. The oft-cited experiments of Fischer (1906) and Kolle (1906) suggested that annihilation of ' paratyphus ' bacilli in broth and milk culture by heating for 30 minutes at 60° C. or for 5 minutes at 75° C. is by no means certain, and Twiss (1920) concluded that a few paratyphoid bacilli might survive heating in milk at 60 to 65° C. for half an hour. More recent work, however, shows that, provided none of the bacilli escape the full shock by drying on the walls of the container, they are infallibly killed by a few minutes' exposure to temperatures between 58 and 60° C. The classic experiments regarding the fate of ' Paratyphus ' bacilli in meats during cooking were somewhat disconcerting in their results. Uhlenhuth and Hübener stated that in sausage meat the bacilli may survive boiling for 2 hours ; Rimpau that infected sausages may lie in water at 95 to 96° C. for $\frac{1}{2}$ to $\frac{3}{4}$ hour without complete destruction of paratyphus bacilli which they contain : there are other reports to a similar effect ; all doubtless depending on the inadequacy of the cooking process, as such, rather than on any special resistance of the bacilli in meat.

General Characters of Growth.

Gelatin culture. The medium is never liquefied. Surface colonies are in most cases circular, translucent, glistening and greyish-white ; in *B. typhosus* a flattened vineleaf form is typical ; in the case of rough variants the same general peculiarities are encountered as on agar. Certain species (*B. paratyphosus B, B. abortus equi*) normally, and others (*B. enteritidis* and *B. suipestifer*) less regularly, grow in surface culture at room temperature as ' slime drop ' colonies, and when confluent the

growth may flow down the surface of the slanted medium to collect at the butt of the tube : in Germany this phenomenon has been regarded as being of diagnostic value.

Agar culture. On nutrient agar, surface colonies of normal races are circular, slightly raised, limited in their spread, and glistening, moist and ' coli-like ' in appearance ; they are in varying degree translucent and vary in colour from greyish-white to brownish-grey. Considerable contrasts in growth vigour occur. *B. abortus equi* is commonly differentiated by the habit of forming a dry wrinkled growth on agar ; irregularities, however, exist. When the plate cultures of *B. paratyphosus B*, *B. abortus equi* and *B. enteritidis* are incubated for 18 to 24 hours at 37° C. and are then allowed to develop at room temperature, there normally forms about each of the more widely separated colonies a complete or partial wall or ring of slimy secondary growth, raised and turbid, and marked by fine radial striæ. Both American and European *B. suipestifer* may show the slime wall fully or partially developed. In Germany considerable attention has been devoted to the phenomenon, particularly in relation to the diagnostic differentiation of *B. paratyphosus B* (' Schleimwall '-positive) from the Breslau bacillus (' Schleimwall '-negative). Opinion as to the value of the test has differed widely and irregularities in both senses have been reported. R. Müller (1925), Elkeles (1926), Knorr (1927) and others have pointed out the influence of the medium, of the area and depth of medium available, of the reaction of the medium and of its salt concentration, on the formation of the slime ring. Gressel (1928) states that *B. sanguinarium* may often be differentiated from *B. pullorum* by its ring-forming activities.

Fermentation Reactions.

Fermentation Reactions of the Group.

Lactose, saccharose, adonitol and erythritol are not attacked : the same statement is usually made with regard to salicin ; but exceptions exist. All types ferment glucose with, in the great majority of cases, evolution of hydrogen and carbon dioxide. Distribution of fermentative action on arabinose, xylose, rhamnose, maltose, trehalose, raffinose, mannitol, dulcitol, inositol and glycerol is irregular ; this irregularity is to a large extent intertypal, but also to some extent affects the individual serological types. With a few exceptions gas is produced in fermented media, the amount varying with the carbohydrate attacked and the organism active ; anaerogenic strains of most of the commonly occurring aerogenic types are known. Various organic acids and their salts are fermented.

Aerogenic and Anaerogenic Fermentation.

The fermentative attack of the Salmonella typically involves a considerable evolution of gas. In two known types alone, *B. typhosus* and *B. sanguinarium*, is this feature regularly absent. In the Glässer, Voldagsen

and so-called Ferkeltyphus strains gas production is somewhat irregular. Similar differences occur in the case of *B. pullorum* (Hadley, Caldwell and Heath, 1919 ; Gressel, 1928). It is usually agreed that *B. paratyphosus A* is less active in gas production than *B. paratyphosus B* and *B. aertryck*. Anaerogenic strains of all the commonly occurring aerogenic types have been reported with varying frequency.

Differential Carbohydrate Reactions of the Types.

The carbohydrates for which differential value has been claimed are : the pentoses, arabinose, xylose and rhamnose ; the disaccharides, maltose and trehalose ; the trisaccharide, raffinose ; the polysaccharide, dextrin ; and the alcohols, mannitol, dulcitol, inositol and glycerol. *Arabinose* is with rare exceptions attacked readily by *B. aertryck, B. paratyphosus B, B. enteritidis, B. abortus equi* and *B. paratyphosus A, B. sanguinarium* and *B. pullorum* and by most of the rarer types (Stanley, Morbificans Bovis, Newport, Reading, Moscow, Derby, Dublin, Tokyo). Between the closely related Suipestifer and Hirschfeld types striking division occurs on the issue of arabinose fermentation : the Hirschfeld strains attack the sugar briskly, while the American and European races of *B. suipestifer* are generally characterized by failure to attack or by late and feeble attack. In the case of the European strains, animal or human in origin, arabinose fermentation—even late acidification of the medium—seems to be rare, though instances of vigorous fermentation are known. In the case of *B. typhosus* a negative arabinose reaction is the rule ; and, according to Miessner and Baars (1927), *B. abortus ovis* also fails to ferment this sugar.

Xylose is almost invariably attacked by all known members of the Salmonella series, save *B. typhosus*, which behaves irregularly, and *B. paratyphosus A*, which is usually regarded as quite inactive. According to White (1926) the Sendai type shares this peculiarity of the serologically related *B. paratyphosus A*.

Rhamnose is fermented by almost all Salmonella types ; the sole known exceptions—those partial—being *B. typhosus* and the Reading type. Ordinarily, that is to say without educative cultivation, *B. typhosus* has little ability to cause acid fermentation of rhamnose media. In culture on rhamnose agar it shows the peculiarity, regarded by R. Müller (1908) as diagnostic, of forming, within the primary growth, a crop of minute secondary colonies or papillæ. The behaviour of the Reading type is somewhat comparable : only one of 10 strains examined by the writer acidified rhamnose peptone-water within three weeks at first trial ; all formed a certain number of papillæ on rhamnose agar ; but these, unlike those of *B. typhosus*, set up vigorous acid fermentation. Bitter, Weigmann and Habs (1926) have used the degree of acidity developed in milk containing 0·5 per cent. of rhamnose for differential purposes. After 15 hours' incubation in this medium, methyl-red is added to the cultures, to indicate by the red or yellow colour assumed the high or low acidity produced : among strains examined, only the Breslau bacillus (*B. aertryck*) and

B. paratyphus N_2 (Moscow type) were found to determine the red indicator change ; *B. paratyphosus B*, *B. enteritidis*, *B. suipestifer* and *B. paratyphus* N_1 (Hirschfeld type), setting up less acidity, were marked by a yellow coloration. Pesch and Maschke (1928) have suggested further refinement based on the differing ability of Breslau, Gärtner and Paratyphus B bacilli to utilize rhamnose as a sole source of carbohydrate. The medium employed was : washed agar in water—1 litre ; K_2HPO_4—1 gm. ; $MgSO_4$ —0·5 gm. ; NaCl—0·02 gm. ; $FeSO_4$ and $Ca_3(PO_4)_2$—traces ; NH_4Cl —1·63 gm. ; with 2 gm. of rhamnose added after autoclaving. On this medium almost all Breslau strains showed growth in 20 hours, while the great majority of Paratyphus B and Gärtner strains failed to develop. The writer has used rhamnose in peptone-water with litmus indicator to effect a slightly different grouping : in this medium Hirschfeld's bacillus fails to produce appreciable acidity before the third day of incubation at 37° C., whereas, apart from the Typhosus and Reading types (negative) and the American Suipestifer and Glässer-Voldagsen types (irregular), all the types, including human and porcine strains of European *B. suipestifer*, cause vigorous fermentation in 24 hours.

Maltose is apparently attacked by all types save *B. pullorum*. Krumwiede and Kohn (1917) noted that even in the case of *B. pullorum* late acidity might develop in maltose peptone-water, and German descriptions suggest some irregularity in the attack of this organism on maltose.

Trehalose. The value of trehalose for differential purposes was pointed out by Koser (1921), who found that *B. suipestifer* (American) was unable to ferment this substance, whereas *B. enteritidis*, *B. paratyphosus A*, *B. paratyphosus B* and ' animal paratyphoid B ' (presumably *B. aertryck*) did so readily. Confirmation of Koser's observation is given by Jordan (1923).

Raffinose. Statements with regard to raffinose fermentation by the common Salmonella species are somewhat at variance, but the weight of opinion would seem to be that acid and gas are not formed in any case. The special interest of raffinose lies in its application by R. Müller (1908, 1914) to the differentiation of *B. paratyphosus B* from other Salmonellas ; particularly from *B. breslaviensis* (*aertryck*). Müller found that in plate cultures of *B. paratyphosus B*, made on raffinose agar, there developed, in the course of several days of incubation, numerous small secondary colonies (papillæ or Knöpfen), springing up within the primary colonies ; these papillæ were absent or scanty in corresponding cultures of Breslau and Gärtner strains. Müller's statement has been confirmed by Penfold (1911 and 1912), Zeller (1922), Saisawa (1913) and Nuck (1927). Almost all observers have reported irregularities.

Dextrin. Almost every possibility with regard to differential Salmonella attack on dextrin has at one time or another been alleged. Until reasonably pure samples of the constituents of ordinary dextrin have been individually examined no definite conclusion can be reached.

Dulcitol is almost always readily attacked by the members of types other than *B. typhosus*, *B. pullorum*, European *B. suipestifer*, the American hog-cholera bacillus. *B. paratyphosus A*, the Sendai type and *B. sanguinarium*, by their delayed but usual activity, occupy an intermediate position. Strains of *B. aertryck* and *B. enteritidis* which do not ferment dulcitol are occasionally encountered. Lack of dulcitol-fermenting vigour on the part of American *B. suipestifer*, noted by Savage (1908) and emphasized by Jordan (1917), Krumwiede, Kohn and Valentine (1918) and others, like that exhibited by European *B. suipestifer*, supplies a usual means of differentiating these forms from the closely related Hirschfeld type, which attacks this substance readily. The negative behaviour of the American and European Suipestifer types towards dulcitol is less constant than the corresponding behaviour towards arabinose; almost all observers have noted late, or occasionally even rapid, attack on dulcitol by individual strains. It is sometimes possible, after repeated subculture in dulcitol broth, to isolate, from strains originally inactive, races attacking dulcitol readily. The Glässer-Voldagsen strains seem to attack dulcitol slowly.

The differential value of *inositol* was recognized by Weiss and Rice (1917). The substance is fermented by the great majority of strains of *B. paratyphosus B* and *B. aertryck*, but not by *B. typhosus*, *B. paratyphosus A*, *B. abortus equi*, *B. suipestifer* (American and European), the Hirschfeld type or *B. enteritidis*. The fermentation set up by paratyphoid B and Aertryck strains varies greatly in vigour as regards both acid and gas production, and the phase of acidity may be transient. Among the rarer types vigorous attack has been observed in the case of the Reading, Derby, L, and Morbificans Bovis strains, while the Glässer-Voldagsen, Newport, Thompson, Moscow, Dublin and Tokyo and Sendai types have been found inactive; Stanley type strains have behaved irregularly in our tests.

Mannitol. Apart from rare instances of suppressed or delayed fermentation on the part of individual strains, mannitol is readily fermented by all Salmonella bacilli save the Glässer-Voldagsen races (Pfeiler, 1919; Pfeiler and Engelhardt, 1919; Manteufel, Zschucke and Beger, 1921) and *B. abortus ovis* (Miessner and Baars, 1927).

Glycerol. So far as the important Salmonella types are concerned it seems that acid formation in glycerol media is slight and that, on this point, no tangible intertypal differences have been observed. There is, however, another type of fermentation in which certain types engage actively. The work of Stern (1916), Zeller (1922), Lütje (1924), Miessner (1925) and Miessner and Baars (1927) shows that certain forms (*B. paratyphosus B*, *B. breslaviensis* and *B. enteritidis*), now termed 'Stern-positive', cultured in fuchsine-sulphite-glycerol-meat-extract broth, cause the indicator to assume a deep lilac-red colour in the course of a varying period of incubation; other forms (*B. typhosus*, *B. paratyphosus A*, *B. suipestifer*, Glässer-Voldagsen bacilli, *B. abortus equi*, Hirschfeld's

bacillus, *B. sanguinarium*, *B. abortus ovis* and *B. coli*) determine at most a pale-pink coloration. It would seem that the positive Stern reaction is due to the formation of aldehyde products of fermentation ; the pale pink sometimes seen in the negative tests is due to generation of traces of acid : no contrast is revealed by a litmus indicator.

The Sequence of Phenomena in Litmus Milk.

Litmus milk forms one of the most valuable of known media for preliminary valuation of Salmonella strains. No member of the group sets up sufficient acidity to occasion clotting ; all, however, cause the indicator to change, within 24 hours at 37° C., from the violet of neutrality to a more or less definite pink—varying from lilac to clear pink—registering the formation of acid in small amount. This is doubtless due to the presence of traces of readily fermentable and glucose-like sugar. Further events divide the Salmonella series into two groups. One group is composed of *B. paratyphosus B*, *B. aertryck*, *B. enteritidis*, *B. abortus equi*, the American and European Suipestifer types, the Hirschfeld type, the Newport, Stanley, Reading, Derby, Moscow, Dar-es-Salaam, L, and Thompson types and *B. morbificans bovis*. In the case of these forms the acid indicator undergoes the so-called ' chameleon ' change ; in the course of a few days (usually 2 to 5) it assumes the blue colour of alkalinity, the change usually beginning at the air-exposed surface of the medium and extending downwards. In some cases the medium may be seen to pass through a stage of neutrality. Associated with the development of definite alkalinity, due to the alkaline products of slight attack on the milk proteins, there occurs, in the course of time, a clearing of the hitherto turbid medium with the formation of a deposit. The second group of strains is composed of *B. paratyphosus A* and the Sendai type, to which are possibly to be added the Glässer-Voldagsen strains (Pfeiler, 1919 ; Pfeiler and Engelhardt, 1919) and *B. abortus ovis* (Miessner and Baars, 1927). In the case of these organisms the acid reaction developed during the first 24 hours is maintained and has often been described as permanent. This, however, is not, at least in all cases, strictly true. Definite as it is for practical purposes, the contrasts of behaviour between *B. paratyphosus A* and the ' chameleon ' forms of the first group is a matter of time rather than of quality. After the lapse of a very variable period, occasionally as short as seven days, usually several weeks, the indicator as a rule registers a return to neutrality or the development of actual alkalinity (Boycott, 1906 ; Krumwiede, Pratt and Kohn, 1916 ; Jordan, 1917).

Decolorization of Rothberger's Neutral Red Agar.

Neutral red agar (Rothberger) with 0·1 to 0·3 per cent. of added glucose has been employed for differential purposes ; the differentiation which it effects seemingly running parallel with the aerogenic or anaerogenic behaviour of the strains. The gas-forming species such as *B. paratyphosus B* and *B. aertryck*, incubated as shake cultures, cause first fluorescence and then decolorization of the medium. Anaerogenic forms such as *B. typhosus*,

B. sanguinarium and certain Glässer-Voldagsen strains (Manteufel, Zschuke and Beger, 1921; Pfeiler, 1919) and anaerogenic strains of types which normally form gas cause no indicator change.

Fermentation of Organic Acids and their Salts.

Various studies of the action of intestinal bacilli on organic acids have shown that a large number of these substances are fermentable by members of the Salmonella series, and Brown, Duncan and Henry (1924, 1926) have made attempt to utilize certain of them for purposes of type-differentiation. Investigation of a series of open chain acids, used as sodium salts in 1 per cent. solution in nutrient broth with Durham's tubes and phenol red as indicator, showed that, with gas formation and the development of alkalinity as criteria, a certain amount of differentiation of typhoid and A, B and C paratyphoid bacilli and other Salmonellas could be effected by the use of formic, citric and d-tartaric acid. Irregularities in the reactions observed led the workers to adopt the lead precipitation method of Brown (1921) in determining the action of the organisms on the several acids. The test depends on the fact that the lead salts of many organic acids are insoluble, and can, under the considered circumstances, be thrown down, usually in the form of a copious flocculent precipitate, from the unfermented medium by addition of lead acetate to the latter. Where the sodium salt of organic acid has been destroyed by bacterial action addition of lead acetate causes only the appearance of a sparse, rapidly settling precipitate consisting mainly of carbonates. By the use of this test and citric, d-, l- and m-tartaric, fumaric and mucic acids, Brown, Duncan and Henry observed the distribution of fermentative activity indicated in Table I.

TABLE I.

	Citrate.	d-Tartrate.	l-Tartrate.	m-Tartrate.	Fumarate.	Mucate.
B. paratyphosus A ..	−	−	−	−	−	−
B. paratyphosus B ..	+	−	+	−	−	+
B. paratyphosus C ..	+	+	−	+	−	−
B. suipestifer	+	+	−	+	+	−
Salmonella, type G ..	+	+	−	+	+	−
,, ,, Reading	+	+	−	+	+	+
,, ,, Mutton	+	+	+	+	+	+
,, ,, Newport	+	+	+	+	+	+
,, ,, Stanley	+	+	+	−	+	+
,, ,, Dar-es-Salaam	+	−	−	−	+	+
,, ,, Derby	+	+	+	+	−	+
B. enteritidis	+	±	±	±	+	+

+ = fermentation ; − = no fermentation.

TABLE II. 'Typical' behaviour of Salmonella types in certain differential tests.

	Slime-wall Test.	Formation of H_2S: Lead Acetate Test.	Litmus Milk (3rd to 7th day).	Stern's Test: Glycerin Fuchsine Broth.	Bilter's Test: Rhamnose Milk.	Fermentation of Carbohydrates in Peptone-Water Solution.										
						Glucose.	Arabinose.	Xylose.	Rhamnose. 24 hours.	Rhamnose. 72 hours or later.	Maltose.	Trehalose.	Mannitol.	Dulcitol.	Inositol.	Salicin.
Sanguinarium	†/—	†	a/alk	—	Yellow	A	A	A	A/—	A	A	.	A	A	—	—
Pullorum	—	†	a	—	Yellow	AG/A	AG/A	AG/A	AG/—	AG	—	.	AG/A	—	—	—
Typhosus	—	††	a/N/alk	—	Yellow	A	—	A	—	—	A	.	A		—	—
Paratyphosus A	—	—	a	—	Yellow	Ag	Ag	—	Ag	Ag	Ag	Ag	Ag	Ag (*l*)	—	—
Enteritidis	††/—	†††	Alk	†††	Yellow	AG	AG/A	AG	AG	AG	AG	AG	AG	AG	—	—
Dublin	·	†††	Alk	·	·	AG	AG (*l*)	AG	AG	AG	AG	.	AG	AG	—	—
Derby	·	†††	Alk	·	·	AG	AG	AG	AG	AG	AG	.	AG	AG	AG	—
Moscow	—	†††	Alk	·	Red	AG	AG	AG	AG	AG	AG	.	AG	AG (*l*)	—	—
Abortus Equi	†††	—	Alk	—	·	AG	AG	AG	AG	AG	AG	.	AG	AG	—	—
Abortus Ovis	—	·	a	—	Yellow	AG	—	·	·	·	AG	.	AG/—	AG	·	·
Dar-es-Salaam	—	†††	Alk	·	·	AG	AG	AG	AG	AG	AG	.	AG	AG	·	Ag (*l*)
Paratyphosus B	†††	†††	Alk	†††	Yellow	AG	AG	AG	AG	AG	AG	AG	AG	AG	Ag/A/a	—
Stanley	—	†††	Alk	·	·	AG	AG	AG	AG	AG	AG	AG	AG	AG	a/—	—
Aertryck	—	†††	Alk	†††	Red	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG/Ag/a	—
Newport	—/†	†††	Alk	·	·	AG	AG	AG	AG	AG	AG	AG	AG	AG	—	—
Reading	—	†††	Alk	·	·	AG	AG	AG	—	—	AG	AG	AG	AG	AG	—
Tokyo	·	†††	Alk	·	·	AG	AG	AG	AG	AG	AG	.	AG	AG	—	—
Thompson	·	†††	Alk	·	·	AG	—	AG	AG	AG	AG	.	AG	AG	—	—
European Suipestifer	††/†/—	†††	Alk	—	Yellow	AG	—	AG	AG	AG	AG	—	AG	—	—	—
American Suipestifer	†/—	—	Alk	·	·	AG	—	AG	AG/—	AG	AG	—	AG	—	—	—
Hirschfeld	—	†††	Alk	—	Yellow	AG	AG	AG	—	Ag	AG	.	AG	AG	—	—
Glässer-Voldagsen	—	†	a	—	Yellow	Ag/A	Ag/A	Ag/A	A/Ag	A/Ag	Ag/A	.	—	Ag/A	—	—
Morbificans Bovis	—	†††	Alk	·	·	AG	AG	AG	AG	AG	AG	.	AG	AG	AG	—
Sendai	—	—	a	·	·	AG	AG	—	AG	AG	AG	.	AG	Ag (*l*)	—	—
Bombay	·	†††	Alk	·	·	AG	AG	AG	AG	AG	AG	.	AG	AG	.	—
L	—	†††	Alk	·	·	AG	AG	AG	AG	AG	AG	.	AG	AG	AG	—

Explanation: †††, ††, †, indicate, as the case may be, varied vigour in slime-wall formation, in H_2S production, or in reddening Stern's medium; A or a, strong or slight acidity; Alk or alk, strong or slight alkalinity; N, neutrality; and G or g, vigorous or moderate gas formation in fermented media. — indicates negative behaviour; (*l*), activity delayed till the third to fifth day; frequent variant conditions are separated by bars. Statement of positive fermentation reactions refers, for the most part, to an incubation period of 24 hours; of negative reactions to one of several days.

In the case of *B. typhosus* and *B. enteritidis* results were irregular. In the case of the fumarate 96 hours' incubation was allowed ; in other cases 48 hours was found adequate. Study of the attack of *B. suipestifer* on citric acid showed that the products of fermentation are acetic and succinic acids and carbon-dioxide. It was also found that all the types tested attack fumaric acid, reducing it to succinic acid ; it is the further fermentation or non-fermentation of this latter substance on which the differential findings in the lead precipitation test depend.

Formation of Hydrogen Sulphide in Cultures of Salmonella Bacilli.

Using, as indicator, soluble salts of lead and iron added to the culture medium, Orlowski found that *B. typhosus*, unlike *B. coli*, produced H_2S and darkened the medium. Similar differentiation of *B. paratyphosus B* (H_2S positive) from *B. paratyphosus A* (H_2S negative) was reported by Sacquépée and Chevrel (1905). These observations have been upheld by Burnet and Weissenbach (1915), Hollande and Bouverie (1915), and other workers. Both *B. enteritidis* and *B. aertryck*, like *B. paratyphosus B*, form H_2S vigorously, but *B. abortus equi* resembles *B. paratyphosus A* in its lack of activity (Krumwiede, Kohn and Valentine, 1918 ; Zeller, 1922, and others). The lead acetate test separates European *B. suipestifer* and Hirschfeld's bacillus, which both produce H_2S, from American *B. suipestifer*, which, with rare exceptions, is inactive in this respect (Jordan and Victorson, 1917 ; Krumwiede, Kohn and Valentine, 1918 ; TenBroeck, 1918 ; Spray, 1920 ; White, 1926). The Glässer-Voldagsen-Ferkeltyphus races all produce H_2S, though somewhat slowly (Pfeiler and Engelhardt, 1919 ; White, 1926). *B. sanguinarium* and *B. pullorum* appear to behave irregularly. Stanley, Newport, Reading, L, Derby and Morbificans Bovis strains all darken lead acetate rapidly.

Serology.

The adequate serological comparison of two Salmonella strains is not a comparison of two things of fixed state, but of two very definite systems of variation—of two life-cycles—the corresponding phases of which must, so to speak, be brought into apposition, each to each, before coincidence or contrast can be truly established. The first question to be considered is therefore that of variation.

Serological Variation and the Salmonella Antigens.

On p. 88, three distinct types of antigenic change—H-form — O-form variation, smooth-form — rough-form variation, and specific phase — non-specific phase oscillation—have been briefly noted : these will be considered in sequence.

H-form — O-form variation : H Antigen and O Antigen.

The essential fact revealed by the studies of Weil and Felix (1920) and others is that the typical Salmonella—in the language of Weil and Felix, *the ' H ' form*—presents two distinct classes of antigens, each stimulating

in vivo its own particular antibodies with which it exclusively reacts. One class of antigens, *the ' H ' labile, or coarsely flocculating antigens,* are more or less severely damaged at temperatures above 65° C. and, when heated at 100° C. or when treated with alcohol or dilute acid, are totally destroyed, ceasing to function in serological tests or as antigens ; their development is inhibited by culture on certain media, notably the phenol-agar medium of Braun ; with the corresponding agglutinins they determine agglutination of the bacilli in large, loose and readily dispersible clumps.

The second class of antigens, the *' O ', stable* or *finely granulating antigens*, resist unimpaired prolonged heating at 100° C. and treatment with alcohol and dilute acid ; are unaffected by phenol agar culture ; and with their special antibodies and where their behaviour is not masked by collateral reaction of the ' H ' elements, decide agglutination of the bacilli in fine compact clumps which survive vigorous shaking of the medium. There can now be no doubt that the labile and stable antigens respectively correspond with the ' flagellar ' and ' somatic ' antigens differentiated by the studies of Smith and Reagh (1903), Beyer and Reagh (1904), Orcutt (1924) and Yokota (1925) : localization of labile and stable antigen in flagella and bacillary bodies respectively has been prettily demonstrated by Arkwright (1927[2]), who, watching under the microscope the agglutination of living motile cultures of *B. typhosus*, found that, while a serum acting on the labile factor alone threw the bacilli into loosely knit immobile groups, an ' O ' serum caused by agglutination compact clumps which continued to tour the field of vision. Now, in nature there occur, and in the laboratory there arise, Salmonella races in which the labile factor is permanently or temporarily reduced or lacking ; these races are non-motile, exactly simulate in their serology cultures in which the labile component has been destroyed or inhibited, and are the *O forms* of Weil and Felix.

The following relevant points may be noted : (1) In the reaction of the ordinary motile culture with its antiserum, the agglutination determined by the flagellar reagents usually exceeds in titre and out-paces in rapidity that due to the somatic reagents ; through the range of dual reaction agglutination is complete, and clumps of both types may usually be discerned ; beyond the range of somatic reaction, clumping is as a rule incomplete and is of the loosely floccular type. (2) Uncontaminated floccular clumping occurs under the action of antisera containing no effective somatic agglutinins, for instance, in the case of sera sapped of such agglutinins by saturation with ' O ', heated or alcohol-treated bacilli ; the clumping observed under these conditions is seldom complete, even at the lower serum dilutions, owing presumably to presence of a variable number of aflagellate bacilli. (3) Ordinary non-motile ' O ' cultures, phenol-agar cultures, and cultures treated with alcohol or heated at 70 to 100° C. show, with any of the corresponding antisera and with those of undamaged flagellate bacilli, a purely granular type of clumping which tends to be complete almost to the titre limit. (4) The somatic and flagellar agglutinins

appear to be entirely independent ; in suitably arranged absorption tests either type may be withdrawn from the serum, leaving the other type intact. (5) Though the ' O ' condition, agglutinatively, absorptively and immunogenically devoid of O antigen, seems often to be the product of sudden loss, all transition stages in the reduction of the labile factor are to be encountered ; first to disappear is the power to condition coarse flocculation of the bacilli, next the ability to absorb appreciable amounts of flagellar agglutinins, and last, the power to stimulate these *in vivo*. The view has been developed by Weil and Felix (1920) and Weil (1921) and supported by Olitzki (1926) and others that the stable elements are solely responsible for the phenomenon of complement fixation in the presence of specific serum ; that the labile antigens do not fix complement. The present writer (White, 1926) concluded that while the stable complex is assuredly the dominant complement-binding factor, the labile elements probably also play a minor role in the process ; this too is the opinion expressed by Hofmeier (1927[1]) and Springut (1927). The actual decision is beset with difficulties. Hofmeier (1927[2]) and Springut have upheld the assertion of Felix and Olitzki (1926) and Olitzki (1926) that the O (endoplasmatic) antibodies alone exhibited bactericidal power ; they believe, however, that both the ectoplasmatic (flagellar) and endoplasmatic (somatic) antibodies exert a marked effect in forwarding phagocytosis.

Specific-Phase—Non-specific-Phase Variation.

It has long been known that cultures of Salmonella bacilli are subject to wide variation as regards the specificity of their agglutinative reactions. Marked differences in the specificity of freshly isolated cultures, vicissitudes in that of individual strains, contrasts in the range of reaction of races sprung from single colonies were all known to occur : the laboratory culture was more or less generally regarded as a heterogeneous collection of units presenting all possible states between type specificity and wide non-specificity. An important step towards an understanding of these variations and the composition of the Salmonella culture was made by Andrewes (1922). Examining plate cultures of *B. paratyphosus B*, *B. aertryck*, *B. suipestifer*, and the Newport and Hirschfeld types, Andrewes found that all presented two serological types of colony which he termed, in respect of their qualities, specific and unspecific (or ' group '). The bacilli of *specific colonies* and their immediate issue agglutinated significantly only with sera of their own type and especially with sera prepared against specific races of that type. The bacilli of *unspecific or group colonies* and their direct descent agglutinated vigorously with sera—particularly group race sera—of all the types named. So far as his observations went Andrewes found that ' specificity ' and ' unspecificity ' were alternative states unlinked by intermediate forms. The truth of these observations was confirmed by Topley and Ayrton (1924) who called the forms respectively ' type ' and ' group ', by Bensted (1925), by Krumwiede, Cooper and Provost (1925), and by White (1925, 1926), who introduced

the term ' phase variation ' to cover the phenomenon ; following on some desultory observations by Sakai (1925[1 & 2]) and Hayashi (1926[1 & 2]) the observations of Andrewes and White have been methodically redescribed by Aoki and his co-workers in a veritable *feu de joie* of papers (Aoki, 1927 ; Aoki and Takayanagi, 1927 ; Aoki and Hayashi, 1927 ; Aoki, Sakai and Hayashi, 1927[1 & 2]; Hayashi, 1927[1, 2, 3, 4, 5 & 6]).

Certain irregularities are mentioned on p. 112 : here we are concerned only with variation within the strain. Important points with regard to the qualities and behaviour of the contrasting forms or phases may be summed up as follows : (1) so far no feature of cell or colonial morphology has been observed to differentiate the serological phases ; (2) the phase changes affect only the labile, flagellar or ' H ' antigen (White, 1926) ; the somatic antigens—both smooth and rough—are the same in both phases ; ' O ' forms and cultures in which the labile antigen has been destroyed show no phase differentiation ; (3) the antisera of the contrasting phases show that selective action on the homologous race which is to be expected, but each phase rouses a minor development of agglutinins for the other—agglutinins which, when ' isolated ', are usually ineffective in clumping the immunizing race ; it is clear, as Andrewes pointed out, that the specific phase contains a trace of group antigen, the non-specific phase a little of the specific substance ; (4) the agglutinative contrast between the phases is correlated with marked cross-absorptive inefficiency (Andrewes, 1925 ; White, 1925) ; exhaustion of specific phase serum with the non-specific form is as a rule more readily accomplished than the reverse proceeding ; in some cases effective exhaustion of high titre non-specific phase serum with the specific race appears to be impracticable ; it is a probable deduction that the non-specific phase is rather less pure than is the specific ; (5) apart from a few special cases, noted on p. 90 and again on p. 112, and provided any influence due to community of the somatic complex is withdrawn, the type specificity of the specific phase is absolute ; it agglutinates only with the specific phase agglutinins of its type ; (6) the non-specific phases of the various types cross agglutinate readily with the non-specific phase agglutinins of other forms, though they are by no means, as Bensted (1925) believed, agglutinatively or absorptively identical ; (7) the writer (White, 1926) attempted to differentiate the phases by means of the complement-fixation test and concluded that it is just possible to do so ; the difference is so ill-defined that the method can have no practical application ; (8) comparative bactericidal tests with phase antisera reveal no tangible differences.

The *transmutability of the phases* and the measure of their constancy have been examined by Andrewes (1922), Topley and Ayrton (1924) and White (1925). As a rule each phase gives rise to the other more or less readily, the race passing back to the mixed condition commonly encountered in laboratory cultures. Reversion may be demonstrated either by use of a suitable serum acting only on the phase in question and applied to serial subcultures or by careful sifting of colonies appearing in

platings made at each stage : detection of incipient impurity may be difficult. First subcultures made in agar or in broth usually present, to all practical intent, pure growths of one phase or the other ; even at this stage, however, impurity may be detected, due in many cases to selection of colonies of mixed nature. How soon an originally pure race will yield the second form it is impossible to foretell : in many cases this occurs at the second or third subculture ; in the majority, mixture is patent by the eighth–tenth subculture ; but some races may be transferred 20 to 30 times without evident change. Phase cultures in cold store cannot perhaps be relied on as a continuous source of pure subcultures, but under these conditions phase constancy is far greater than at room temperature or 37° C. Tendency to change in fluid culture is more marked than in agar culture. The view of Topley and Ayrton (1924[2]) that it is usually easier to obtain the specific phase from a non-specific race than to make the reversed isolation, is probably to be accepted. Scott (1926[1]) dealing with stubbornly non-specific races of his Thompson type of Salmonella was able to stimulate appearance of the specific phase by addition of non-specific phase serum to the medium. While colonies containing both types of bacilli are often encountered and yield colonies of their component phases, no clear evidence of races in which the qualities of both phases are truly blended is yet forthcoming. Topley and Ayrton (1924[2]) have stated that specific phase races of *B. aertryck* grown in broth at 37° C. lose something of that specificity of agglutinative behaviour which they exhibit when incubated in the same medium for 16 hours at 22° C. ; that at 37° C. they develop an antigen (' X ' antigen) which decides clumping to variable titre with Newport-type antisera. The writer (White, 1926) was unable to detect the alleged anomaly : the point requires further study ; it is possible that rough antigen or some totally unspecific factor is concerned.

Smooth-form—Rough-form Variation and the Corresponding Antigens.

The *growth changes* associated with rough variation in the Salmonella group conform to the pattern of v. Lingelsheim's (1913) and Arkwright's (1921) original descriptions. The typical rough colony, in contrast to the smooth moist circular and raised parental form, is flattened, spreading and irregular in outline ; its surface appears frosted and dry, and under the hand-lens or microscope exhibits a varying degree of granularity, ridging, facetting or other irregularity which justifies the term rough ; the rough colonies have the granular consistence of semi-solidified grease and may show increased opacity. Considerable variation occurs from culture to culture and from colony to colony in the degree of development of these characteristics of the rough state. This to some extent depends on the existence of transition forms in which the essential antigenic change associated with roughness is incipient or partial. The culture medium, too, exercises an important influence : a dry medium tends to emphasize the peculiarities of growth ; so also does bile-salt agar ; on phenol agar the

outward signs of roughness may be largely suppressed. Occasionally races are encountered in which a smooth growth habit is associated with serological roughness (White, 1926).

Agglutination by electrolytes and normal serum. In distilled water the vast majority of rough Salmonella races form stable and evenly dispersed suspensions; dispersal and stability are but little affected by presence of 0·2 per cent. of NaCl; in 0·4 per cent. salt solution a proportion of races slowly clump and precipitate; and in 0·85 per cent. salt solution the great majority are so speedily agglutinated that specific agglutination tests cannot, under ordinary circumstances, be properly performed; at higher saline concentrations, though some zonation may occur, 'spontaneous' agglutination is still more rapid. Salts of the heavy metals cause agglutination in disproportionately low concentrations. In broth culture this sensitiveness to the precipitating action of salts is registered by heavy deposition, often so complete as to leave the supernatant almost clear. Under certain circumstances rough Salmonella bacilli agglutinate rapidly with normal serum though the titre attained (100 to 400) seldom achieves that (1,000 to 2,000) usual in like conditions in the Colon and Dysentery groups. The writer (White, 1927, 1928, 1929) has shown: (1) that the phenomena of agglutination in the presence of salts and normal serum depend in the first place on absence of a carbohydrate-containing substance (see p. 116) the hydrophile character of which determines the stolid insusceptibility of the smooth organism to unspecific agglutinating agencies; in the second place upon the presence of certain hydrophobe, salt- and serum-precipitable substances which dominate the surface of the rough organism; (2) that, without modification of the essential serology of the rough organism, one class of such hydrophobe substances, the bacterial 'lipoids', may be removed by treatment of the bacilli with absolute alcohol, rendering them insensitive to salt in physiological—and sometimes in twice physiological—concentration, though usually failing to prevent eventual clumping in 3·4 per cent. NaCl solution or eventual agglutination by normal serum; (3) that, however, both these latter events may be inhibited by subjecting the bacilli to 65 to 70 per cent. alcohol, a procedure accompanied by some change in specific serology; (4) that the agglutination of rough Salmonella cultures by normal serum occurs to an appreciable extent only when the saline concentration of the reaction mixture falls to 0·4 per cent. or less and is conditioned by reduction of the solution stability of the serum-globulin; that the phenomenon depends on some interaction of the substances extractable by absolute and 70 per cent. alcohol and euglobulin.

Specific serology. Though the cultural and physical peculiarities of the rough Salmonella have been repeatedly described (Q forms of v. Lingelsheim, 1913; Grote, 1913; Gildemeister, 1916[1&2]; Wagner, 1920; Bumke, 1924) no clear statement of a serological contrast between smooth and rough races appears to have preceded that of Schütze (1921), who not only recorded the existence of agglutinative differences between

the two forms, but also drew attention to a strange ' cosmopolitanism ' of agglutination of rough variants to rough sera ; to a broad non-specificity of reaction involving types serologically distinct in the smooth state. The truth of these observations, questioned by Krumwiede, Cooper and Provost (1925), was confirmed by White (1925, 1926), who showed that the essential feature of rough change is an alteration of the stable somatic complex of the organism with development of a new and characteristic heat- and alcohol-resistant antigen, in which the labile flagellar elements are not of necessity involved. This thesis was in turn confirmed by Goyle (1926[2], 1927) and Arkwright (1927[1]), who abandoned an earlier view (Arkwright and Goyle, 1924) that the smooth form and rough form coincided respectively with the O form and H form of Weil and Felix ; isolated descriptions of this type of serological modification have been presented by, among others, Gruschka (1923) and Fürth (1923).

The main points of serological interest are : (1) The typical rough variant is characterized negatively by failure to respond to the ' O ', somatic agglutinins of the corresponding smooth serum and by failure to rouse *in vivo* agglutinins acting on the somatic antigens of the smooth organism ; positively it is marked by special and considerably stable somatic antigens which react with the homologous rough antiserum to cause clumping of the bacilli in fine granules (often condensing in soft flakes readily to be differentiated from those of flagellar agglutination), and forming a muddy readily dispersible deposit quite unlike the sandy sediment seen in smooth somatic agglutination. (2) There is no evidence that in rough change the labile flagellar elements suffer qualitative modification ; in diphasic species the specific and non-specific phases are clearly recognizable in company with roughness ; in certain cases, however, roughness may be consistently associated with loss of flagellar antigen. (3) In the majority of cases smooth and rough races are sharply differentiable by absorption tests performed in both directions ; in certain cases the rough form shows sufficient remnant of smooth substance to influence the result. (4) The agglutinative cosmopolitanism of rough Salmonella variants with rough sera extends throughout the Salmonella group, but apparently not very far beyond its limits ; in many cases absorption tests suggest that the rough somatic antigens of different Salmonella types are almost identical, but irregularities, as yet unexplained, are to be encountered, even within the individual type. (5) The complement-fixation test differentiates sharply between smooth and rough bacilli, but the latter show in complement-binding behaviour the same intertypal non-specificity which marks their agglutinative reactions (White, 1926).

Manipulation of rough cultures for serological study. In serological tests spontaneous agglutination by salts has usually been held in abeyance by reducing the saline concentration of the reaction mixture to 0·2 per cent. of NaCl (Arkwright), and, where the labile flagellar factors or much heated bacilli are under study, this is the only procedure possible : but, owing to the agglutination with normal serum thereby conditioned, readings

below dilution 400 are rendered insignificant; and under all other circumstances tests should be performed, in presence of 0·85 per cent. of NaCl, with bacilli treated with *absolute* alcohol (30 to 60 minutes at 50 to 55° C.).

Nature of rough variation. Following up the work of Reimann (1926) on the pneumococcus, the writer (White, 1927, 1928, 1929[1]) has shown that the essential serological event in rough change is loss of a carbohydrate-containing serologically active substance, a haptene, individually non-antigenic, which constitutes the 'O' receptor (as distinct from the entire 'O' antigen) of the smooth organism (see p. 116). The reactive bacillary surface exposed by, or developed after, this loss seems to be essentially lipoid and protein: it is apparently this protein which decides the agglutinative peculiarity and non-specific serology of the rough variant.

A differential chemical test for roughness. When to 3 c.cm. of a dense suspension of rough bacilli in distilled water is added 0·5 to 1 c.cm. of Millon's reagent, and the mixture is boiled, the bacilli coagulate in dense clumps and assume a deep red-pink colour (positive reaction); under the same circumstances smooth bacilli remain dispersed and their colour does not deepen beyond an ochre tint (White, 1929). The Molisch test performed under identical conditions with emulsions of smooth and rough bacilli is usually most strongly positive in the case of the former.

Conditions favouring rough change and reversion to smoothness. Preserved on laboratory media all cultures eventually develop, but with very varying rapidity, an admixture of rough growth, which tends, with subculture, to supplant the smooth parent form: many of the old classic strains are known to us only in the rough form. Frequent subculture in fluid media and long standing in such media promote roughening, and it is seldom difficult to obtain rough variants by such treatment; on inspissated egg media the tendency to change is apparently reduced. Burnet (1927, 1929), Webster and Burn (1927) and others have observed roughening under bacteriophage action and others have noted similar change in cultures exposed to homologous smooth antiserum. Schütze (1921) and White (1925), who worked with old laboratory cultures, concluded that roughening was probably irreversible, but later work shows that true recovery of smoothness may, under certain conditions and at least in certain cases, occur (Burnet, 1927 and 1929—bacteriophage action; Jordan, 1926—rapid subculture in veal infusion broth, pH 7·4; animal passage, see p. 143).

Mucoid Variants and their Serology.

Both in platings from pathological material and from laboratory cultures, colonies of Salmonella bacilli may assume the so-called 'mucoid' form. W. Fletcher (1920) describes such variants of *B. paratyphosus B* and *B. aertryck* from fæces of carriers and convalescents; Thjotta and Eide (1920) and Sonnenschein (1926) write of mucoid *B. paratyphosus B* from urine and blood respectively; Nelson (1927) of similar forms of

' *B. paratyphi* '—presumably *B. enteritidis*—from guinea-pigs ; the writer has isolated typical mucoid races of *B. paratyphosus B*, *B. enteritidis* and *B. abortus equi* from laboratory cultures. There is probably a connection between tendency to develop the fully mucoid growth habit and ability to produce a ' Schleimwall ' at room temperature (see p. 94). Sonnenschein (1926) and others have observed development of mucoid variants under bacteriophage influence. The constancy of mucoid races is variable : some few for a time show no sign of reversion, but the majority quite early yield in subculture a varying number of normal, non-mucoid colonies which usually breed true. Most observers have described the mucoid cells as coccoid in morphology : the units, without individual encapsulation, are held in a common intracellular matrix. In broth and peptone-water culture some (Fletcher, Nelson) found their strains motile, others (Thjøtta) non-motile ; we have seen both conditions.

Serological behaviour. Two of Fletcher's three mucoid races failed to agglutinate with, or to absorb, the agglutinins of the normal race ; Thjøtta's strain agglutinated very slowly with, but eventually was clumped to the full titre of, ordinary paratyphoid B serum ; Sonnenschein speaks of his strains as inagglutinable ; Krumwiede, Cooper and Provost (1925) concluded that mucoid strains possessed definite agglutinative and absorptive individuality ; Nelson on the contrary states that his mucoid strains behaved in a normal manner. Our own studies (White, 1926, and later observations) suggest that as a rule, when grown on solid media, mucoid races react very slowly and feebly as ' O ' forms ; coarse floccular agglutination is absent. The vigour of their reaction to ' O ' agglutinins is increased by heating the suspensions to 100° C. before use : cultures grown in broth may or may not show floccular agglutination. The antisera of mucoid races have no more vigorous action on the variant than have ordinary antisera and are completely exhausted of agglutinins by normal cultures.

Transmutation of Type.

It has been repeatedly alleged that Salmonella types, though they normally maintain their individuality, may change one into the other. We proffer a few examples from the recent literature. Seligmann (1927) following up earlier work (Seligmann, 1926), describes isolation, from several old Gärtner cultures, of races, all described as ' zakig ' in colonial form, which, unclumped by Gärtner serum, agglutinated with Paratyphus B serum ; Köhlisch (1918) asserts similar mutation of typhoid bacilli to the serological Paratyphus B type, the colonies of the variant races being ' Weinblattformig ' : in both cases, though the qualities of the test-sera can only be assumed, there is reason to suspect that the observations were based on nothing better than Schütze's phenomenon of cosmopolitan agglutination of rough variants. The statements of Tey (1927), regarding transmutation among the types Paratyphi B hominis, Freiburg and Breslau, rest, in our opinion, on ignorance, not on knowledge, of the phenomena of bacterial variation. Space limitation forbids analysis of

Tey's observations, but it is clear that phase changes, possibly also H-form—O-form variations, played a part in the phenomena described : one of two 'Freiburg' (Aertryck) strains declared to have delivered *B. paratyphi B. hominis* as variant is the Göttingen strain of Wichels (1924)—an unquestionable *B. paratyphosus B* strain: here the original diagnosis of type, based possibly on the non-specific phase, was doubtless in error. Pesch (1927) fed 6 mice on a typical Gärtner strain ; of these, 3 died after lapse of 3 to 4 weeks, and from all were isolated, not Gärtner, but Breslau bacilli. Pesch argues against the possibility of intercurrent Breslau infection, and in favour of actual change in the mouse body from the Gärtner to the Breslau type : had the bacilli chosen any other type for this *volte face* of character, had the experiment been again and again repeated with like result, then the argument for change of type might be more convincing. Such statements, like the suggestion of Wagner (1913) that anaerogenic paratyphoid bacilli were derived from *B. typhosus* isolated from the same case, can only be regarded as records of opinion, not as proof. The Salmonella types have doubtless been derived by radical processes of change from a common source ; definite, seemingly irreversible, changes have occurred, and probably occur : of loss variations we know something and such events seem to have played a part in the phylogeny of the group ; of progressive changes we know nothing ; they have not yet come under controlled experimental observation. The idea that the Salmonella types merely represent resting-points in a system of inter-labile variation has nothing to support it ; no available instance of transmutation stands longer scrutiny than the reading of its record demands. The Salmonella types are seemingly discrete entities, elementary species ; a few successful, many abortive ; each traversing its little orbit of individual modification along the lines its ancestry has laid down. That at certain stages of their cycle some of these forms approach one another more or less closely no more destroys their several individualities than does the morphological similarity of two mammalian ova damn the specificity of their divergent products.

Application of Serological Methods to Differentiation and Identification.

The Agglutination Test.

As a means to the solution of the group, the agglutination test has passed through vicissitudes of esteem. For long the simple test appeared to afford an adequate means for recognition and differentiation of *B. typhosus, B. paratyphosus A, B. enteritidis* and the Paratyphoid B group : that sharp contrasts could by this means be effected within the limits of the last named was doubted. We now know that the existence of various overlapping and linking forms destroys much of the diagnostic finality of the test as regards these four classic divisions of the genus, and that on the other hand intense intertypal contrasts can be demonstrated within the old Paratyphoid B group.

Methods. For general purposes the writer prefers bacillary suspensions prepared in water or saline from *moist* agar growths : in research work these form the only ' plastic ' material for study ; there is here less tendency to variation than in broth—in phase studies an important point —and to accumulation of unspecific serum-precipitable substances in the suspending medium. No labour should, however, be spared, where the flagellar antigens are in question, in encouraging motility by broth culture and in selecting from platings actively motile races for study. For preservation of suspensions chloroform is excellent ; formalin is to be avoided. Of the many methods of preparing bacilli for study of somatic antigens, treatment with alcohol at 50 to 55° C. is the best. The manipulation of rough variants is discussed on p. 107. The isolation of specific and non-specific races of diphasic species calls for note : the picking of colonies for study may be made at random, but is most successful when assisted by such preliminary tests as knowledge and ingenuity suggest and the bulk of the colony allows. For such tests dense emulsions are prepared from the chosen colonies in drops of water on a slide : these are now tested with the non-specific phase agglutinins of a heterologous type, the serum used being either naturally or by absorption free of somatic agglutinins for the type under test ; instantaneous (usually partial) agglutination indicates a non-specific (or else a mixed) colony ; absence of agglutination a specific (or perhaps an ' O ') colony. In differentiating Aertryck, Stanley and Paratyphosus B colonies, Suipestifer sera are valuable and may usually be employed neat ; for separating Suipestifer colonies, Aertryck and Paratyphosus B antisera are excellent ; from Table III the suitable sera in each case may be ascertained. Fuller tests, which rule out possible errors of the preliminaries, are made with 16 to 18-hour cultures from the chosen colonies with serial dilutions of (i) a heterologous non-specific phase serum such as used in the first trials, i.e. without action on the bacillary bodies, and (ii) an homologous specific phase serum robbed of non-specific phase and somatic agglutinins. Satisfactory non-specific phase races react only with (i), true specific races only with (ii), ' O ' races with neither, and mixed cultures with both. For incubation of agglutination tests the writer favours a temperature between 48 and 52° C.—that of 55° C. often employed represents the limit of safety so far as flagellar antigen is concerned.

The Differential Agglutinative Reactions of the Salmonella Types.

The *reactions of the somatic antigens* divide the known types more or less frankly into seven ' groups ' (White, 1926) : (i) Enteritidis, Tokyo, Dublin, Moscow, Typhosus, Sanguinarium, Pullorum and Dar-es-Salaam ; (ii) Aertryck, Paratyphosus B, Stanley, Reading, Derby and Abortus Equi ; (iii) Newport and Morbificans Bovis ; (iv) Hirschfeld, Suipestifer (American and European) and Thompson ; (v) Sendai and Paratyphosus A ; (vi) Bombay ; (vii) L. Minor and variable somatic cross agglutination occurs between groups (i) and (ii) and occasionally

group (v); and between groups (iii) and (iv). The special 'O' relationships of *B. typhosus*, *B. enteritidis*, *B. sanguinarium* and *B. pullorum* have been the subject of many studies (Weil and Felix, 1920; Gruschka, 1920 and 1923; Fürth, 1923; Goyle, 1926[2], 1927; Smith and TenBroeck, 1915[1&2]; Edington, 1924; Krumwiede, Cooper and Provost, 1925; White, 1926; Takayanagi, 1926; Lubinski, 1928). Most German authors have regarded the 'O' reactions of Gärtner, typhoid and fowl typhoid bacilli as identical; Goyle, however, considered that marked differences existed in this respect between Gärtner and typhoid bacilli. The writer's studies, though considerable, have not been entirely conclusive. In his experience all the four organisms named above react with Enteritidis, Pullorum and Sanguinarium sera to a like (somatic) titre; with some antityphoid sera the reactions, in accordance with the German statements, are also indistinguishable; with others, however, the somatic reaction of the homologous organism, as Goyle found, far excels that of the other forms. We suspect that *B. typhosus* possesses a somatic factor, of variable importance, in excess of those held by the other forms named.

The *flagellar agglutinations* of the monophasic types and of the specific phases of the diphasic forms permit ready differentiation of all these species with the following exceptions: they fail to separate the specific phases of the Stanley and Bombay types or to differentiate either from *B. typhosus*; they make no contrast between the specific phases of the Newport and Reading types or between *B. paratyphosus A* and the specific phase of the Sendai type; between the specific races of the Sendai type and the Dar-es-Salaam type; between the Enteritidis, Dublin, Derby and Moscow types *inter se*, or between these and the specific phase of the Tokyo type; between the specific phase races of Hirschfeld's bacillus, American *B. suipestifer* and Glässer's bacillus. Apart from these exceptions the known motile types, including all the commonly occurring species, may be differentiated in the sharpest possible manner. The non-specific phases of the diphasic types and the permanently non-specific forms all cross-agglutinate more or less extensively with the flagellar agglutinins of non-specific phase or mixed phase antisera in general. Nevertheless, two main groupings appear: (i) Paratyphosus B, Aertryck, Stanley, Bombay, Newport and Tokyo; (ii) Suipestifer (American and European), Hirschfeld, Reading, Morbificans Bovis, Sendai and Thompson.

From all lines of study it is possible, by systematic application of the agglutination test to the main antigenic variables of the smooth Salmonella to achieve the following division of the group: Paratyphosus B, Stanley, Bombay, Aertryck, Newport, Reading, Morbificans Bovis, American Suipestifer (+ Hirschfeld and Glässer's bacillus), Thompson, L, Tokyo, Sendai, Paratyphosus A, Typhosus, Derby, Enteritidis (+ Dublin and Moscow), Dar-es-Salaam, and Sanguinarium (+ Pullorum). The European Suipestifer (+ Voldagsen) and the Binns strains stand off from American Suipestifer and Aertryck respectively. This differentiation depends on the assumption that all the variants are available for study.

The Agglutinin-Absorption Test.

Broadly speaking the reciprocal test performed with two bacillary suspensions and their antisera may give one of three results, statement of which is simplified by the preface that—all assertions to the contrary notwithstanding—each suspension will always, in adequate dosage, remove all appreciable *specific* agglutinative action on itself either on the part of its own or any heterologous antiserum. The possibilities are: (i) that each suspension exhausts the serum of the other (absorptive identity); (ii) that while one suspension exhausts the serum of the other, this second fails to exhaust the serum of the first (one-sided absorptive relationship; superstrain-substrain relationship of Schütze, 1921); (iii) that neither suspension exhausts the serum of the other (bilateral absorptive contrast). Until recently it was deemed adequate in testing for difference and identity to prepare antisera for the two organisms in gross culture and to test the homologous agglutinative power of each serum before and after treatment with a considerable amount of the heterologous organism. It is now realized that this simple type of test, successful though it often is, is no better than a shot in the dark; in all crucial tests the procedure must be analytic; somatic antigen must be matched against somatic antigen, flagellar antigen against flagellar antigen, each phase against the corresponding phase. Several forms of laxity, studiously to be avoided, have frequently marred the use of the test. Often test protocols show imperfect absorptive treatment of the serum; Bock (1906) and Schiff (1922), who literally makes a virtue of the vice, offer examples: in all cases dosage should be pressed until the value of the serum ceases to fall or falls below the accepted limit of observation—usually serum dilution 50 or 100: in deciding the adequacy of saturation, addition of bacilli to the limit of their agglutination is no valid criterion. The practice of testing the absorptive effect at one serum dilution only is bad; the approximate titre of the absorbed serum should be determined. Strains of a single type, granted agreement in phase, in ' H ' and ' O ', and smooth or rough condition, react in a practically identical manner with any type serum; once their identity is established—rigidly established—they are interchangeable. Substitution of strains in absence of these searching preliminaries is unwarranted.

Another fallacious mode of applying the test is to attempt to apportion a series of strains A, B, C, D, E, F . . . , between two types, X and Y, by testing them against X serum before and after saturation with Y, and against Y serum before and after treatment with X; were there but two types to consider this method might suffice; in practice, it has led to confusion: the inherent danger of this procedure, instanced in the papers of Bainbridge and O'Brien (1911) and Jordan (1917), has been emphasized by Schütze, who urges the necessity of comparing each strain directly with the type organism. Though it fails as a test for identity, this form of treatment, systematically carried out, seems to yield information regarding the relative antigenic composition of the types: when

X serum absorbed by Y is found to retain agglutinins for X and Z, then it seems probable that X and Z possess in common an antigenic component lacking in Y. Starting with this working hypothesis, White (1925, 1926), paying respect to phase and to flagellar and somatic antigens, tested a series of types against the homologous and heterologous antisera before and after saturation of these with single and variously combined heterologous cultures. The results showed considerable consistency and it seemed possible to apply to certain types relative antigenic formulæ from which their behaviour in any particular serological circumstance might be deduced. The system of symbols assigned is presented in Table III. Schiff (1923), without consideration of phase, made similar examination of the Paratyphus B, Breslau and Suipestifer types; his conclusions, so far as they go, are identical.

Among points of note which may be extracted from Table III are the following: (1) Certain types differ serologically from certain others only in negative features and their antisera are exhausted by these superior forms. The full antisera of the serologically inseparable forms, Pullorum and Sanguinarium, are exhausted by the Enteritidis, Dublin, Tokyo and Typhosus types; those of the Enteritidis and Paratyphosus A types by the Tokyo and Sendai types respectively; those of European Suipestifer (and *B. voldagsen*) by American Suipestifer and Thompson; those of the Binns strains by Aertryck. (2) The fully representative sera of certain types, while resisting exhaustion by any single heterologous form, succumb to particular combinations of other types: Stanley type sera are exhausted by combined action of Paratyphosus B (or Aertryck) and Typhosus; Typhosus serum fares little better under joint action of Enteritidis and Stanley; Sendai type serum yields to Paratyphosus A with Suipestifer; Newport serum to the trio of types, Morbificans Bovis ('O' agglutinins), Aertryck (non-specific 'H' agglutinins) and Reading (specific 'H' agglutinins); Reading serum is almost destroyed by the system Aertryck, Newport and Suipestifer; and so on. It is seen that a number of types depend for individuality on the varied groupings of a limited number of antigenic components: in these cases, as in those listed under (1), preparation of monospecific (monovalent) sera, in the sense of Andrewes (1922), is out of the question. (3) The full sera of a number of types cannot be, even approximately, exhausted by any combination of known heterologous forms; these are: Paratyphosus B, Aertryck, Morbificans Bovis, Moscow, Derby, Dublin, Thompson, and, as a pair, American Suipestifer and Hirschfeld. (4) The American Suipestifer and Hirschfeld strains show only relatively small and quantitatively variable absorptive differences most marked in the case of the non-specific phases; from the first-named type, Glässer's bacillus is indistinguishable.

The Complement-Fixation Test.

From the recent studies of Weil and Felix (1920) and others, the broad fact emerges that the complement-fixation test effects a grouping coincident

TABLE III. *Relative Antigenic Constitutions of Salmonella Types.*

Modified and Simplified from the Scheme presented in Medical Research Council, Special Report Series, No. 103.

| *Meta-Salmonella Types.* | *Monophasic Series :* | | *Diphasic Series.* | | |

with that suggested by the agglutination reactions of the 'O', somatic antigens (see p. 111). Most workers agree that the common types fall into the, by this means inseparable, pairs, Enteritidis and Typhosus, Paratyphosus B and Aertryck, Suipestifer and Hirschfeld, and Paratyphosus A; the writer has traced the correlation between 'O' antigen and complement-binding activity through several other types. Certain workers, such as Dean (1911) and Hecht-Johansen (1923), have alleged the possibility, by refined technique, of differentiating *B. paratyphosus B* and *B. aertryck* by this test: whether bacterial extracts, such as used in Dean's experiments, render available other differential complement-binding components than are supplied by the surfaces of whole bacilli it is impossible to say; the flagellar antigens can afford but little handle for differential purposes. The specific carbohydrates (see Precipitation Reactions) imitate closely the complement-fixing behaviour of the types from which they are derived.

Precipitation Reactions of Certain Derivatives of Salmonella Bacilli.

Some reference must be made to studies, in imitation of the precipitation and so-called thermo-precipitation tests of Ascoli and Valenti and Ascoli performed with the antisera of various Salmonella bacilli and with a variety of extracts of the organs of rabbits dying of Salmonella and other infections: the aim being to develop a method for recognition of Salmonella-infected meats. The main contributions are by Rothacker (1913) and Reinhardt, who regarded the test as fairly specific, and Patzewitsch and Isabolinsky (1913) who encountered wide non-specificity of reaction. Kölln (1921) maintains that the test is of value in the diagnosis of 'calf-paratyphus'. We mention the matter for the reason that determination of the Salmonellar or non-Salmonellar causation of canned-food-poisoning must come along these lines. Interest at present centres on the reactions of certain non-protein, carbohydrate-containing, 'soluble specific substances' and the proteins with which they are associated in the bacilli (Zinsser and Parker, 1923—*B. typhosus;* White, 1927, 1928, 1929[1]—various Salmonella types; Landsteiner and Furth, 1928—*B. typhosus, B. enteritidis* and *B. paratyphosus B;* Happold, 1928; Day, 1928; Branham and Humphreys, 1927; Ecker and Rimington, 1927). Clearly defined are the specific carbohydrates, closely comparable to those of the pneumococcus, which, highly resistant to heat, to acid and to tryptic digestion, and to the best of our belief without antigenic value, react precipitatively in high dilution with the antisera of the whole organisms: in the nature of their compact indispersible precipitates, in the range of their cross reactions, in their failure to react to rough antisera they declare themselves unequivocally as the 'O' *receptors* of the smooth bacillary bodies. The readiest means of effecting their separation is to extract the bacilli with hot dilute acetic acid (White,

1929[1]). With regard to the various other precipitatively active substances which have been isolated it is difficult to speak comprehensively. Landsteiner and Fürth have extracted with hot 75 per cent. alcohol a protein showing wide group relationships, which is probably identical with a substance isolated by the writer under like circumstances from rough variants and which may play a part in the somatic serology of the rough organism ; they also describe a second protein factor serologically distinct from the former. Neither of these proteins reacts appreciably with the antiserum of the whole smooth organism, though each vigorously to its own antiserum.

Acid Agglutination of Salmonella Bacilli.

Michaelis (1911, 1915) claimed that his acid-agglutination test (for technique see Vol. IX) permitted differentiation of *B. typhosus* from the A and B paratyphoid bacilli on the one hand, and the colon and dysentery types on the other. Knowledge of the value, limitations and phenomena of the test has been extended by Beniasch (1912), Eisenberg (1919), Küster, Lange and Pothoff (1921), Arkwright (1914, 1928), Schütze (1920) and Gouwens (1923) ; the writer, working in part with Dr. D. Kalić has examined the relation of serological variation to precipitability by acids. The facts as they at present appear are as follows : (1) Smooth, motile *B. typhosus* presents an agglutination optimum in the region of $[H^{\bullet}]$ $3{\cdot}6 \times 10^{-5}$, while the corresponding races of *B. enteritidis, B. paratyphosus A, B. paratyphosus B, B. aertryck* and most other diphasic types show most vigorous reaction at $[H^{\bullet}]$ $1{\cdot}38 - 2{\cdot}9 \times 10^{-4}$; within this latter series, phase differences where they occur have little appreciable influence on the acid-agglutination optimum. (2) The Stanley type is peculiar in that, in harmony with its serological behaviour, its specific phase reacts in the acid agglutination test like *B. typhosus*, its non-specific phase like *B. paratyphosus B ;* in a trial with the Newport type we have noted a somewhat similar division of affinities. (3) These various smooth flagellate cultures may or may not show a feeble secondary optimum in the region of $[H^{\bullet}]$ $1{\cdot}1 - 2{\cdot}2 \times 10^{-3}$; in the case of smooth aflagellate cultures this feeble and irregularly occurring agglutination at high hydrogen ion concentrations is alone seen. (4) Rough cultures, flagellate and aflagellate are clumped over a wide range extending downwards from a vigorous optimum at $[H^{\bullet}]$ $1{\cdot}1 - 2{\cdot}2 \times 10^{-3}$ to $[H^{\bullet}]$ 2×10^{-4} or less ; treatment of the rough bacilli with alcohol before test does not materially alter their reaction to acid. The vigorous acid agglutination of smooth motile bacilli in low hydrogen ion concentrations is doubtless to be ascribed to the flagella ; the inert behaviour of non-flagellate, smooth bacilli, to the acid-insensitive specific carbohydrate, and it is probably only where this is in part dislodged by acid hydrolysis that acid agglutination of these forms occurs. In the rough organism, disappearance of the carbohydrate factor apparently leaves the somatic proteins exposed to the agglutinating action of acid.

The Occurrence of Salmonella Bacilli in Nature other than the Diseased Host.

There exist two opposing views as to the position of the Salmonella group in nature. That developed by Uhlenhuth and his school, and recently re-expounded by Uhlenhuth (1925), is that the Salmonella group —and more particularly the comprehensive Paratyphus B group—has widely colonized nature, living and inert, in a purely saprophytic manner ; that from this saprophytic foothold morbid infection of man and animals by *certain* potentially pathogenic races from time to time occurs. The contrary view, which probably exists, albeit largely unexpressed and untested, in the minds of most workers in this country, where the work of Bainbridge (1911) and Savage (1908, 1909, 1910, 1918) has determined the trend of opinion, is that the Salmonella group as a whole is essentially pathogenic and depends for its indefinite propagation on a more or less closely connected sequence of actively invaded hosts, from which external nature becomes sporadically and abortively contaminated. It seems proved that Salmonella bacilli may occur in the intestine of the ostensibly healthy *pig ;* as to their frequency and type adequate information is lacking, but the probability is that most are of the Suipestifer type ; many have noted the presence in the pig of atypical ' Paratyphus-like ' bacilli. In spite of the known liability of *cattle,* occasionally of *horses,* and more especially of their young, to Salmonella diseases, search in the intestines and organs of healthy members of these species, and of *sheep,* has given almost entirely negative results. In all these animals, as in pigs, bacilli presenting some, but incomplete, cultural resemblance to true Salmonellas are often found. There are no records of cultivation from healthy *cats* and *dogs,* but from dogs suffering from such diseases as distemper and rabies isolation of organisms of this group has been achieved or alleged.

It has long been known that mice and rats, wild and tame, and other laboratory rodents frequently harbour organisms of the Salmonella group in their intestines and organs. In the *rat, B. enteritidis* is encountered almost exclusively and often in a high percentage of animals ; Meyer and Matsumura (1927) report *B. aertryck,* which is far rarer, from rats trapped in San Francisco ; Kerrin (1928) speaks of a Suipestifer-like organism—probably European Suipestifer—from a pigsty rat. In *mice, B. aertryck* and *B. enteritidis* occur with almost equal frequency ; it is almost impossible to maintain a stock free from the taint. The *guinea-pig* is almost equally subject to so-called latent infection with *B. aertryck* and *B. enteritidis,* and the Reading type has been isolated under like conditions ; in *rabbits* the condition is far less frequent. A claim by Heuser to have isolated Paratyphus B bacilli from the intestinal contents of geese appears to be the only alleged instance of Salmonella saprophytism in *birds.* There exist many records of the isolation of ' Paratyphus B ' bacilli from *healthy persons* (' normal carriers ') and from those suffering from unrelated disease—records which, to all appearance, do not relate to a carrier condition established by generalized or localized infection of the body

proper (Conradi, Küster, Marmann, 1906 ; Hübener and Viereck ; Prigge and Sachs-Müke, 1909; Rimpau ; Aumann, 1910) ; against the generality of the findings of some of these observers stand the negative results of O. Meyer, G. Seiffert (1909) and Sobernheim (1910) and—at least in this Country—general laboratory experience.

Turning to occurrence of Salmonella bacilli outside the living host, it may be said that almost every *foodstuff, beverage* or condiment, not actually lethal to the organisms, may convey such an infection. Isolation of these bacilli from foodstuffs under no suspicion of having caused disease—even from samples of food consumed without ill result—has often been effected or alleged ; from sausages, sausage-meat, Hackfleisch, milk, &c.: to dilute these observations there exist the negative results of other extensive investigations. From *water, B. paratyphosus B* has been several times reported ; *B. paratyphosus A* more rarely. How far all the claims are admissible is doubtful. Though the *fly* is an accepted and obvious agent in the spread of enteric infection its role in the dispersal of paratyphoid and food-poisoning bacilli is far less demonstrated than assumed—the assumption finding support in the more numerous records of isolation in the case of *B. typhosus;* the claims of Nicholl (1911) regarding *B. paratyphosus B,* of Torrey (1912) regarding *B. paratyphosus A,* and of Cox, Lewis and Glynn (1912) with regard to a Gärtner-like organism all leave much to be desired in the way of proof ; Horn and Huber (1911) reported negative findings, and the writer failed to isolate paratyphoid bacilli from 8,000 flies taken in camp and hospital latrines in the Tidworth district of Salisbury Plain during the period 1916–18. Graham-Smith found that flies experimentally infected might harbour *B. typhosus* and *B. enteritidis* for about a week.

Salmonella Infections of Man.

Occurrence in Human Disease.

Apart from the Sanguinarium, Pullorum, Abortus Equi, Abortus Ovis and Glässer-Voldagsen types, which have not up to the present been isolated from human sources, all the known Salmonella types have been found associated with, and certainly or probably causing, disease in man.

Classification of the Human Salmonelloses.

The Salmonella infections of man may be divided into : (1) *Salmonella fevers* (typhoid, paratyphoid), generalized infections of subacute course, but which may show septic localization. (2) *Salmonella gastroenteritis* (' food-poisoning '), intensely irritant infections of the gastro-intestinal tract which may with similar acuteness be extended to the body as a whole. (3) *Localized pyogenic infections.*

A and B Paratyphoid Fevers.

Typhoid apart, these fevers, caused respectively by *B. paratyphosus A* and *B. paratyphosus B,* are the most important in the first category of

human Salmonelloses. While both fevers may conform in entirety to the typhoid type in span, character and severity, they tend to deviate from this pattern in several ways : in absence of ' stepladder ' temperature rise ; in remissions and intermissions with lack of continua ; brevity of course and mildness of symptoms ; furthermore in the B type fever there may be a varying display of gastro-enteritic symptoms at onset. In typical cases there is, as in typhoid, a characteristic macular or papulo-macular ' rose spot ' eruption (roseola) ; the pulse is slow relative to the temperature and is unstimulated by atropin ; marked leukopenia with disappearance of eosinophiles is usual. The incubation period appears to vary between 4—even 2—and 12 days or more. The case mortality varies greatly with conditions (1 to 16 per cent.), figures in the region of 2·5 to 3·5 per cent. being usual ; it tends to be higher in the B than in the A fever and lower in both than in typhoid.

There have been many instances of *simultaneous or overlapping infections* with A and B paratyphoid bacilli and *B. typhosus* in varied combination. Castellani (1915) gives an account of a case in which all three organisms were isolated. Cases of mixed infection with Shiga or Flexner dysentery bacilli, of association with cholera, relapsing fever, meningococcal meningitis, measles and malaria have been frequently or occasionally reported.

The *bacteriological findings at autopsy* vary only in detail. Normally spleen, liver, bone-marrow and mesenteric glands, often also the contents of the small and large intestine, the lesions of the intestinal wall, pus from abscesses and exudates, yield the bacilli in culture. Almost invariably the organism is present in the gall-bladder, where Suzuki found it at 47 of 48 autopsies ; the same author, confirming Pick, refers to a somewhat similar affinity for the seminal vesicles.

Carriers of A and B Paratyphoid Bacilli.

The pivot of the carrier problem in paratyphoid, as in typhoid, is the individual who, having suffered the disease, continues in convalescence, beyond it, or indefinitely, to harbour the infecting organism and to discharge it to the exterior. According to the duration of his activity in this respect he is classed as a convalescent (acute, temporary) or a chronic carrier : some have selected three months, some six months, dating from onset of fever, as the arbitrary limit of the convalescent carrier state. While other and interesting types of carrier exist, those in which the bacilli are discharged in fæces, in urine, or in both, present the main problem for discussion. Undoubtedly the doctrine of the ubiquity of *B. paratyphosus B* and of its frequent occurrence as a saprophyte in the human intestine, ridiculing all hope of bacteriological control, prevented, in the case of paratyphoid B carrier, any immediate parallel to the illuminating studies inspired by Koch's practical campaign against typhoid.

Apart from scattered observations and the studies of Gaehtgens (1907), Hilgermann (1910), and, in the case of paratyphoid A, of Grattan

and Harvey (1911), information with regard to the paratyphoid carrier and his condition springs in main from investigations enforced by the Great War (Reibmayr, 1918; Bumke, 1925[1 & 2]; Leishman, 1923). The war-time data relate to an adult male population; information as to the *case and general incidence* of paratyphoid carriers in an ordinary population is scanty and any statement on the latter issue is rendered difficult by that uncertain quantity, the 'normal carrier'. Examining 1,076 persons engaged in the dairy industry in Alabama, Welch, Dehler and Havens (1925) found 39 carriers of *B. typhosus*, 13 of *B. paratyphosus A* and 3 of *B. paratyphosus B*—a carrier total of 55 (=5·1 per cent.); of the typhoid and paratyphoid A carriers, 18 and 5 respectively were urinary carriers; paratyphoid B bacilli were isolated only from fæces. MacMaster (1926) reports on 49 cases of paratyphoid B at Dover: 15 (4 males and 11 females) became temporary carriers; 1 woman, *æt.* 64, a chronic carrier. As regards the war-time data: of 27,330 enteric convalescents received at Spa during the first three years of hostilities, 1,609 (6 per cent.) were carriers, previously known or later detected; while through the stated period the estimated case incidence of typhoid carriers fell from 4·4 to 0·9 per cent.—a fall attributed to the salutary effect of vaccination—that of paratyphoid carriers rose from 4·4, through 2·8 to 7·6 per cent.; the increase was assumed to be due to more efficient examination. Owing to inadequate data regarding the composition of the total convalescents, separation of the figures for A and B paratyphoid was impossible (Bumke, 1925[1]). Reibmayr (1918) found that 30 per cent. of A and B paratyphoid sufferers still showed the organisms in their fæces at defervescence, and in 9·6 per cent. they continued to be excreted through eight fever-free weeks or longer. The figures gathered by Leishman (1923) from the records of the Enteric Convalescent Depot at Addington Park, relate to the case incidence of the chronic carrier judged on a six months' term: of 837 A and 1,425 B type convalescents, 26 (3·1 per cent.) and 43 (3 per cent.) respectively became chronic carriers: this against 16 (2·93 per cent.) of 546 typhoid convalescents. Restrictedly fæcal carriers far outnumber those who discharge the bacilli in urine alone, but in a considerable number of cases collateral fæcal and urinary excretion of the organism occurs. The contrast in frequency of fæcal and urinary excretion is especially marked in the case of chronic carriers; the Addington Park records show: chronic typhoid carriers, 15 fæcal and 1 urinary; chronic A type carriers, 21 fæcal and 1 urinary; chronic B type carriers, 43 fæcal and 1 urinary.

Often *onset* of carrier excretion is deferred till late in the post-paratyphoid period: Bumke (1925[1 & 2]), to whom the reader is referred for full analysis, gives instances in which A or B type bacilli were not detected in fæces or urine, as the case might be, before the 11th to 22nd week after onset of paratyphoid.

The general *duration* of the carrier state demands rather fuller consideration. This can be best given by summary of the data of Bumke

(1925[1]). In the case of 105 fæcal and 20 urinary carriers of *B. paratyphosus A*, 62 (59 per cent.) and 13 (65 per cent.) respectively ceased to excrete the bacilli within 3 months of paratyphoid onset; 24 (23 per cent.) and 4 (20 per cent.) respectively ceased excretion within the next period of 3 months, leaving 19 (18 per cent.) stool carriers and 3 (15 per cent.) urinary carriers in the chronic category. In 13 cases fæcal excretion and in 1 urinary excretion persisted beyond the sixth month and in most of these the bacilli were still observed over a year. Of 338 fæcal and 40 urinary carriers of *B. paratyphosus B* bacteriologically recognizable while at the Carrier Station, 110 (38 per cent.) and 24 (60 per cent.) became 'negative' in the first quarter-year after onset of fever. In 34 (12 per cent.) instances excretion of the bacilli was shown to continue in the stools, in 4 (10 per cent.) in the urine, for a period longer than 6 months, but a large number of men had to be discharged uncured, and of these the majority probably belonged to the chronic class; against this last item had to be balanced 373 men sent to the station as carriers, but there found bacteriologically negative; these had to be accounted among the 'cured'. Bumke concluded that in the first 3 months 60 to 70 per cent. of carriers became free of the organism, that a further 10 to 20 per cent. were cured in the second 3 months; and that some 20 per cent. became chronic carriers. Among 109 chronic carriers—so accounted by a standard of 3 months' excretion—Reibmayr (1918) found that 31 ceased discernible activity before the end of the first half-year; during the second half-year no single carrier passed the test of 10 successive examinations.

Causes of the carrier state. The concept of the chronic carrier who affords a nidus for purely saprophytic multiplication of the organism has been almost completely abandoned; experience is decisive that protracted carrier excretion depends on the existence of chronic lesions, by virtue of which alone the organism maintains its foothold in the body. Forster (1908), speaking particularly of typhoid, drew attention to the association of the fæcal carrier state with gall-bladder disease; in the case of paratyphoid carriers abundant instances of this association have been forthcoming. Such observations pointed to the gall-bladder as the reservoir from which flow of the bacilli appearing in the fæces was maintained, and chronic infection of the gall-bladder was accepted as the essential feature of the carrier state. To this conclusion later study has added some qualification; its inadequacy is indicated by the fact that extirpation of the gall-bladder does not invariably terminate carrier excretion; of which lack of avail Pribram (1912) supplies an example. Reibmayr (1918) and Bumke (1925[1]) have emphasized that during the first year or 15 months of carrier life choleocystitis is rare, but that diffuse pains referable to the liver and to the scattered lesions, lymphomatous foci, pseudo-tubercles, necroses and abscesses therein, are often present; the suggestion is that these points of lingering bacterial activity form the primary feature of the carrier state; that from these bacteriocholia is at first maintained, and that the later choleocystitis, by which the bacilli consolidate their position

in the bile-tract, is a result rather than a cause of the carrier condition. The lesions in the liver may heal, leaving simple, but none the less secondary, choleocystitis, in association with which carrier excretion persists. If before onset of typhoid or paratyphoid disease there exists an inflammatory condition of the gall-bladder, so much greater is the liability of the latter to secondary invasion.

The case of the urinary carrier presents close parallel ; analogous to liver abscesses are abscesses of the kidney, which, discharging into the tubules initiate bacteriuria ; equally comparable with the secondary choleocystitis are secondary pyelitis and cystitis. The question has been repeatedly raised—for instance, by Krause (1921)—as to whether chronic ulcerative or other conditions of the intestine—dysentery, appendicitis and the like—present a nidus for prolonged multiplication of the bacilli ; the weight of opinion is that such is not the case. Reibmayr (1918), arguing against the event, admits that during continuous discharge of the organism in the fæces it may only occur occasionally in duodenal samplings and that it must, therefore, possess some, if limited, ability to multiply in the intestine.

Influence of sex. As in typhoid the case incidence of chronic carriers is definitely greater among women than among men—a difference already indicated by Prigge and Sachs-Müke (1909), and doubtless due to greater frequency of pre-existing gall-bladder disease in women sufferers.

Agglutinative behaviour of the carrier's serum. Bumke (1925[1]) traced statistically the agglutinative response of the paratyphoid carrier through six months or more ; in a considerable percentage of tests performed the findings were negative, the proportion of negative results increasing with duration of the carrier state. Lack, feebleness or disappearance of agglutinins in paratyphoid carriers has frequently been noted ; Hamilton (1910), making the observation, urged the significance of a high opsonic index in diagnosis of the carrier state. Rise and fall of agglutinin titre with periods of carrier activity and quiescence have been reported.

Reibmayr (1918) and Bumke (1925[1]) refer to the occurrence of *tonsillar carriers :* in one case described in detail by Bumke the organism continued in the enlarged tonsils through the fourth month following paratyphoid onset, disappearing after operation. Protracted *discharge of the bacilli in pus* has been occasionally observed (fistula of the arm and periostitis, Gildemeister, 1916 ; otitis media, Klein and Torrey, 1920). Mention must also be made of the so-called ' *porteurs précoces* ' of Sacquépée, individuals who shed the bacilli in their fæces prior to onset of symptoms ; the papers of Hallinan and Roaf (1917) and Fletcher (1917–18) present examples in the case of paratyphoid B, that of Krumwiede (1917) in the case of paratyphoid A. The paper of Krumwiede, just cited, also leads back to the somewhat difficult question of the *normal carrier* of paratyphoid bacilli referred to on p. 122, for it shows that *B. paratyphosus B* holds no monoply in the matter of transient, uneventful passage through the intestine of man. It would seem likely that this occurrence is more or less frequent in any

population acutely exposed to paratyphoid infection, A or B in type. Instances of *mixed carriers* in which A and B paratyphoid bacilli are together discharged or are individually associated with *B. typhosus* or dysentery bacilli are frequent enough; Bumke (1925[1]), Fletcher (1917–18), Rajchman and Western (1917) and Erdheim and Schopper (1916) give excellent examples. Chronic carriers of paratyphoid bacilli occasionally suffer '*auto-infection*'—virtually relapse; for examples see Prigge and Sachs-Müke (1909) and Salomon (1919).

Epidemiology of A and B Paratyphoid.

The importance and geographical distribution of these paratyphoid infections. Though Schottmüller (1900 and 1901) and Brion and Kayser (1902) encountered paratyphoid A at the outset of their Paratyphus studies, the disease was, in Europe prior to the War, little more than a text-book condition: scattered cases of A type infection were reported from Germany, Austria-Hungary and Russia; in France, especially in the South, the incidence seems to have been rather greater; from Britain, during this period we find no other record than that of Windsor (1911, gall-bladder infection associated with fatal tuberculous meningitis): Lehmann (1916) gives an excellent list of European reports. Meanwhile paratyphoid B fever, though unimportant in comparison with typhoid, was abundantly observed throughout Europe. The outbreak of hostilities in 1914 was followed by severe epidemics of typhoid in the French and German forces. At this time, according to Lebœuf and Braun (1917), paratyphoid A was unknown. This latter made its debut in 1915, and by November of that year constituted 92 per cent. of all enteric cases. According to Sacquépée, Burnet and Weissenbach (1915), typhoid, paratyphoid A and paratyphoid B became each in sequence the dominant disease of their group. In the British forces in France both forms of paratyphoid put in an early appearance, but though the B type soon became dominant, it never achieved a tithe of the importance which it assumed in the armies of the other belligerents. Judging from the figures furnished by Torrens (1923), and excluding some 1,000 undifferentiated enteric cases, there occurred in this area, against 2,104 cases of typhoid, 1,082 diagnosed cases of paratyphoid A, and 2,710 of paratyphoid B. There is little doubt that the A type infection was in the main imported by French troops from North Africa, in part by British troops from India.

Turning now to the events in Central Europe and its armies: paratyphoid A appeared about July, 1915; first, we believe, on the West and South-West German fronts, contracted from the French and possibly the Italians; then in the Southern Balkan zone, and finally along the Eastern line, conveyed, it is asserted, by the South Russian troops with which contact had occurred. Seemingly the disease remained to all intents restricted to the military population and to the actual zones of war. Occasional cases cropped up in other parts of Germany. Bumke (1925[1]) estimates that possibly 20,000 to 30,000 cases of paratyphoid A occurred

during the War among the German troops on all fronts ; the disease reached its maximum frequency in the autumn of 1915, and after the autumn of 1915 gradually subsided in importance.

Hardly less interesting in its contrast is the war-time history of paratyphoid B in the German ranks ; appearing during the great typhoid epidemics, it formed at first a small but indeterminate percentage of the diseases of this group ; then rapidly it gained in absolute and relative importance, until by 1916 it dominated the entire picture, and, what is more, it maintained its importance and supremacy through the remainder of the War. Bumke considers the fact that the number of paratyphoid B carriers at the Spa convalescent station was double that of the typhoid carriers a clear indication that *at least* twice as many men suffered from paratyphoid B as from typhoid, and probably many more ; he concludes with the important statement that paratyphoid B, with dysentery and influenza, held first rank in the diseases of War.

In Britain, during the War, A and B paratyphoid bacilli were repeatedly isolated from convalescents returning from France and the East ; a few A type cases and cases of relapse were observed in home hospitals, but the disease did not attack the civil population. Since the War, to judge by the available literature, paratyphoid A seems to have resumed something of its pristine insignificance in Western Europe. The failure of *B. paratyphosus A* to consolidate its position in Europe and to establish itself in the civil population is even more remarkable than its dramatic appearance in the opposing armies. *B. paratyphosus B* on the other hand, seems, at least in certain areas, to have assumed a new importance. Bitter (1921) and Weigmann (1925[1]) present data which indicate a steady rise in frequency, relative to typhoid, of paratyphoid B in Schleswig-Holstein.

Spread of infection. In both diseases the ultimate sources of infection are the active case, the convalescent and the chronic carrier. The mode of conveyance may be by personal contact, by indirect contact through a polluted environment, and by infected food and drinking-water. In the case of paratyphoid A *contact* has been from the first recognized as the main—some have said the sole—mode of spread. Belief in the ætiological identity of paratyphoid B and food-poisoning for long distracted attention from the importance of contact infection in the spread of the former disease ; the experiences of the War effected a radical change of opinion : during that period contact—above all in the trenches—was recognized as the main factor in the spread of both paratyphoid diseases. Secondary cases, mainly due to immediate contact, are common in paratyphoid B epidemics. Established, or indeed probable, instances of *water- or food-borne infection* of the A type are few : the most credible are those presented by Paladino-Blandini (Lehmann, 1916), Erdheim and Schopper (1916), in the case of water, and of Horák (1917), who reports conveyance of infection by water and by food. Paratyphoid B infection carried by water though often suspected does not seem to have been very frequently proven in the bacteriological sense ; proved cases of food- and milk-spread

paratyphoid B are numerous enough. A somewhat unusual observation with regard to *intrauterine infection* with *B. paratyphosus B* is reported by P. Schmidt (1915).

Primary source of infection. The role of the *active case* in the spread of infection calls for little comment though note must be made of *ambulant cases;* in both diseases symptoms may be so slight that the affected person, suffering only passing indisposition readily mistaken for mild influenza, and perhaps associated with some intestinal disturbance, may continue the usual routine of life, moving about among his fellows. Instances have been described by Safford (1913) and Sacquépée (Lehmann, 1916) in the case of paratyphoid A;, and Tietz (1922) mentions the difficulty experienced in keeping in bed some of the sufferers in the Königsberg A type epidemic. McMaster (1926) gives instances of ambulant cases of paratyphoid B. The main agent in the maintenance of paratyphoid disease is the *carrier,* whose origin, character and behaviour has been considered earlier. Quite a number of cases and epidemics have now been definitely traced to carriers and in many other instances a plausible argument has been presented in favour of a similar agency. As clear-cut examples of the part played by carriers of *B. paratyphosus A*, those given by Grattan and Harvey (1911) and Lehmann, Mäulen and Stricker (1915) will serve. Recent and soundly based instances of carrier-spread paratyphoid B infection are supplied by Hamburger and Rosenthal (1918), Williams (1925), McMaster (1926), and Ward (1928), in the case of milk-borne epidemics; less convincing is the argument in that of the outbreaks, also held to be due to milk, described by Linnell (1921) and Soothill and Leggat (1927). The number of outbreaks of paratyphoid which have been actually traced to carriers presumably gives little indication of the true importance of the latter in maintaining these diseases; it is as a rule only in those rarer instances where cases are so grouped or so numerous that enquiries can be brought to a focus that circumstantial evidence of the guilt of a particular individual becomes overwhelming.

B. paratyphosus B *as a Cause of Acute Gastro-enteritis.*

Accepting *B. paratyphosus B* and *B. aertryck* (*breslaviensis*) as distinct bacterial entities—a conclusion long, and even still, contested on the Continent—there still remains the question as to whether *B. paratyphosus B*, in addition to its role in typhoid-like disease, also plays a part in the causation of acute gastro-enteritic food-poisoning. The definitely negative view has been pressed by the German Kiel School, supported by W. Gärtner (1921), who gives an excellent summary and critique, and in this country by Savage. The contrary attitude, relic of original belief in the essential oneness of Paratyphus B and Gastro-enteritis paratyphosa B, has been upheld by Uhlenhuth (1925), Uhlenhuth and Seiffert (1926), Elkeles (1926), and others; these authors, while now admitting the two disease types to be in the main due to distinct races, or to variants of a

single form, maintain that true *B. paratyphi B. hominis* may on occasion set up symptoms, even cause outbreaks, indistinguishable from those due to Enteritidis or Aertryck infection : the main basis of their opinion is afforded by a series of outbreaks, including those of Bainbridge and Dudfield (1911), Prigge (1912), Hamburger and Rosenthal (1918) and Wichels (1924), all deemed due to *B. paratyphosus B*, in which the cases were either mixed in character, some typhoidal, some gastro-enteritic, or else adhered in entirety to the latter type. In the controversy round these outbreaks neither camp has succeeded in carrying conviction to the other ; the heterogeneity of the outbreaks has been suggested ; the rectitude of the bacteriological diagnoses doubted ; even the clinical classification of the cases has been called in question. It would seem, however, that Wichels' conclusion with regard to the outbreak at the Göttingen Eye Clinic stands beyond effective criticism : in 5 of the 10 cases the symptoms were of the true food-poisoning type in onset and course, and representative cultures of the organisms isolated have proved in the writer's hands, as Wichels and others maintained, typical wall-forming *B. paratyphosus B :* however rare its activity in this respect may be, *B. paratyphosus B* has been known to cause acute food-poisoning.

B. aertryck Infections of Man.

B. aertryck, cause of the vast majority of outbreaks and cases of acute gastro-enteritis (food-poisoning, q.v.), demands next attention. Apart from differences in the severity of the cases and variation towards a cholera-like form the disease which it occasions is remarkably uniform : onset is sudden and follows within a few hours (4 to 24) of infection, usually conveyed by food ; the rise and, after the usual febrile course of 3 to 6 days, the fall of temperature may be almost critical ; the picture is dominated by gastro-intestinal symptoms.

As regards the *bacteriology* of the disease ; isolation of the organism is readily made from the stools of the acute attack. Reliable information as to whether there is more or less regularly collateral generalized infection is inadequate. From the organs and heart-blood of fatal cases the bacilli are regularly to be cultured *post mortem*, but it is conceivable that fatalities are restricted to a few cases in which the vicious attack of the organism becomes generalized, or that such infection is a terminal event in the moribund. We note that many reports of positive hæmoculture refer to fatal cases. Uhlenhuth and Hübener (1913), admitting that the organism may occasionally be absent from the circulation, would seem to consider bacilliæmia usual, but such systematic tests as those of Broughton-Alcock (1920), backed by scattered observations (Bitter, 1920 ; present writer), suggest that as a rule generalized infection does not occur. This is supported by the low death-rate (1 to 1·5 per cent. of cases) in a disease of such severity, and by the relatively low agglutinin titres of the blood-sera of convalescents. As a rule the organism vanishes rapidly from the stools, commonly between 8 and 14 days after onset ; but there are

records—how well based on the bacteriological side we do not know—of its excretion for periods of 12 to 14 weeks : *proved* chronic carriers of *B. aertryck* are unknown. *B. aertryck* is relatively seldom found associated with pyogenic conditions in man : but the writer isolated the organism from the pus, taken at operation, of an ischiorectal abscess developing during a fatal attack ; and Brinck (1927) gives instances in which alleged Breslau bacilli were isolated from subphrenic abscess and abscesses of jaw, liver and kidneys.

Of the *epidemiology* of Aertryck food-poisoning we need not speak beyond noting the explosive nature of the outbreaks ; the simultaneity of onset among the cases infected at the same meal ; the rarity of secondary cases ; and the relatively small amount of *exact* information which exists with regard to the probable primary sources of infection. Solly and Henderson (1926) furnish details of a case in which *B. aertryck* infection was associated with fatal disease of unusual symptoms (acute mania) and course (20 days).

B. enteritidis Infections of Man : the Dublin, Derby, and Tokyo Types in Human Disease.

The second role in the causation of acute Salmonella gastro-enteritis is played by *B. enteritidis* Gärtner, which occasions a disease clinically and epidemiologically similar to that caused by *B. aertryck*. Records exist of the rare participation of the organism in disease of other nature. Dean (1911) and Boehm and Bitter (1919) write of cases of cholecystitis in which *B. enteritidis* was isolated from gall-bladder and fæces : Dean's case was apparently of many years' standing and associated with cholelithiasis ; a chronic carrier condition is probably to be admitted. Several times *B. enteritidis* has been reported as cause of meningitis : the instance offered by Stuart and Kirikorian (1926) is valid ; that of Symmers and Wilson (1909) and the two cases of Opitz (1919) are possibly so : that of Pesch (1926) is spurious, the organism concerned being of the serologically distinct Dublin type. It is notable that all these cases were in infants or children. Further, there are a number of claims to have discovered the organism as cause of, or associated with, paratyphoid disease : most notable in this series are the reports of McNee (1921), Gregg and Hayes (1921), Chen-Pien Li and Yin-Yuan Ni (1928), and Rosher and Wilson (1921). How far the bacteriological diagnosis in all these cases has been correct it is difficult to say. The prototype of the *Dublin type* was isolated by Professor J. W. Bigger from the blood of a case of fatal fever supervening after operation to the kidney. With this strain, as already stated, is to be grouped Pesch's (1926) strain from the cerebrospinal fluid of an infant. As causes of human disease the *Tokyo type* ('N' strain of Sakai, 1926) and the *Derby type* (Peckham, 1923 ; White, 1926) are known only from outbreaks of acute food-poisoning in Japan and England respectively.

The Newport Type as a Cause of Human Disease.

The Newport type has come to notice as an occasional cause of outbreaks of acute gastro-enteritis ; the original Newport outbreak and that at Shrewsbury (Savage and White, 1925[2]) were typical explosions in the Aertryck-Enteritidis style. The important epidemic due to this type, reported by Perry and Tidy (1919) from France, was remarkable in that the cases, while individually conforming to the gastro-enteritic type, were scattered over three weeks—virtually the clinic of food-poisoning and the epidemiology of paratyphoid. Hæmoculture gave negative results ; the organism was regularly present in the stools during the acute disease, disappearing therefrom in 50 per cent. of cases by the fourth week, from those of almost all by the seventh, but persisting in one case till the fourteenth week. Carriers (one fæcal and one urinary) of the Newport type have also been reported by W. Fletcher (1917–18) ; in one of the cases discovery of the organism was preceded by an attack of paratyphoid type. The strain ' Paratyphus B_2 ' of Weil and Saxl (1917)—a Newport type organism—seems to have been derived from a case of fever of protracted course in which intestinal symptoms were unmarked ; the organism was obtained from blood and urine but not from the stools. These several observations discover the serological Newport type in a variety of pathogenic roles.

The Types of the Suipestifer-Hirschfeld Series in Human Disease.

European B. Suipestifer *or European* B. paratyphosus C.

This organism plays a dual part in human disease ; it is equally well known as a cause of acute gastro-enteritic food-poisoning, of paratyphoid fever—European paratyphoid C. It has also been isolated from cases of abscess. With regard to *outbreaks of acute gastro-enteritis* it need only be noted that such have only been reported from Europe ; that there are no records of secondary cases and seemingly no instances in which any of the cases assumed a paratyphoid-like or influenzal character ; the form of these outbreaks, the bacteriology and morbid anatomy of the cases resemble those of Aertryck food-poisoning. Of the 25 *cases of paratyphoid nature and of localized pyogenesis* of which the writer has collected records all save one (American) were studied in Europe or Malta, though in one case the infection was contracted before or during voyage from Bombay. It is significant that all the cases have been solitary ; no instance of the grouping of two or more cases is known. A disproportionately large number of the cases, especially those of abscess formation, occurred in infants and children ; and most of those among adults are referable to the period of the War or the immediately succeeding years.

American B. Suipestifer.

Far more rarely than its European congener has this organism been recognized in human disease, but in the few cases known to us it seems to

declare the same varied propensities as the former. Presumably to be ascribed to this type is the food-poisoning strain of Krumwiede, Provost and Cooper (1922); Nabarro, White, Dyke and Scott (private information) speak to two cases, one of joint abscess in a child, the other of sudden onset, without diarrhœa or vomiting, and of highly febrile and delirious course of nine days and fatal termination, in which this organism was isolated from pus and blood respectively. An organism isolated by Hicks (1927) in Shanghai from the blood of a fatal case of paratyphoid fever appears to be of this type.

The Hirschfeld Type.

During the War, and in its several theatres, save the Western, this organism sprang into the limelight of medical interest as cause of an often severe disease of the enteric class, but showing special tendency to septic complication, and, as a rule, relatively little of the intestinal conditions usual in typhoid and paratyphoid A and B. With conclusion of hostilities, records, if not cases, of Hirschfeld type infection, with certain notable exceptions, ceased somewhat abruptly. In Russia, however, during the hunger years of 1921–2, Hirschfeld's bacillus, passing under the title 'Paratyphus N_1 bacillus', appeared as the most important of those secondary invaders which determined the terrible mortality in the epidemics of relapsing fever then raging (Sütterlin, 1923; Hesse, 1924; and Klüchin, 1924; Iwaschenzoff, 1926); with the abatement of the recurrent infection, cases of Paratyphus N infection disappeared. Next there is the notable epidemic Hirschfeld type infection among Javanese coolies in Sumatra described by Bosch (1927), who offers data which suggest that the disease may be endemic in the Dutch East Indies. Finally, there is the case—doubtless one of imported infection—described by Andrewes and Neave (1921) from this country.

Disease of Man due to Certain of the Rarer Salmonella Types.

Second in importance among the 'Paratyphus N' bacilli acting as secondary invaders during the 1921–2 relapsing fever epidemics in Russia were the so-called N_2 strains (*B. paratyphus* C_1 of Weigmann, 1925[1], and the Moscow type of our classification) for which the name Moscow type has been adopted in this article. It seems that in the Russian epidemics the organism was far less responsible for the septic features of many of the cases than was Hirschfeld's bacillus (N_1 bacillus). The prototype of the *Stanley type* and the similar 'Chesterfield' strain, of Peck and Thompson, were isolated in connection with typical outbreaks of food-poisoning; the 'Britton' strain of the same serological type, originating, we believe, from one of Fletcher's (1917–18) cases, was on the other hand isolated from the urine of a convalescent with history of paratyphoid fever and relapse. Up to the present all the few known strains have been drawn from European sources. As to the clinical character of the fatal case, from which, at autopsy, the solitary known

strain of the *Bombay type*, was isolated we have no information; the equally solitary example of the *Dar-es-Salaam type* was derived from the blood of a paratyphoid sufferer; the type names of these two forms indicate their site of geographical origin. The *Morbificans Bovis* and *Thompson types* were respectively observed by Sladden and Scott (1927) and Scott (1926[1]) as causes of food-poisoning in the country; the *L type* strain associated with the Derby type, was obtained by the writer (White, 1926) from a case of similar nature. Remains the *Reading type*, isolated in the first instance by Schütze (1920) from the Reading water supply during investigation of an outbreak of paratyphoid fever (? relation); only once so far has this type been definitely recognised as a cause of human disease; then causing a small outbreak of paratyphoid type, marked by diarrhœa, abdominal pain and roseola (Savage and White, 1925[3]).

Salmonella Infections of Animals.

The part played by the Salmonella in animal diseases is an important one: all domesticated species of birds and mammals—save perhaps carnivores, in which the event seems rare—are in varied degree subject to attack by members of the group; so too the rodent pests of man. With regard to other animals in the wild state very little is known. On the whole the animal Salmonelloses show less individuality than do those of man, and sometimes the Salmonella is but one of a series of invaders which may set up the symptom-pathological picture of the disease. The diseases are, as a class, septicæmic and run an acute, subacute or sometimes chronic course with corresponding differences in the associated lesions.

Spontaneous Salmonella Infections of Rodents.

Rabbits are, of all laboratory rodents, least prone to Salmonella infection. The only clear instance of epidemic infection of these animals of which we are aware is that, presumably due to *B. aertryck*, described by Litch and Meyer (1921). *B. aertryck* is responsible for the great majority of the relatively rare sporadic Salmonella infections of rabbits; *B. enteritidis* accounts for the complement. As agent in the more or less common epizootics and isolated infections among *guinea-pigs*, *B. aertryck* —the *B. pestis caviæ* of American authors—is most frequently to be found. *B. enteritidis* is also often encountered as a cause of spontaneous disease in these animals. *Mice* are particularly subject to Salmonella disease, but so far only two types, *B. enteritidis* and *B. aertryck*, appear to have been isolated from spontaneous infection: *B. aertryck*, which corresponds with the *B. typhi murium* of Loeffler (1892), is perhaps rather more frequent than *B. enteritidis;* to the latter type is to be ascribed the celebrated bacillus of Danysz (1900), isolated from an epizootic among field-mice. So frequently do such infections occur that they have excited little comment in the later literature; Lynch (1922) has described an important epidemic among the laboratory mice at the Rockefeller Institute in which both *B. enteritidis* (Mouse typhoid I) and *B. aertryck*

(Mouse typhoid II) were concerned. In *rats*, *B. enteritidis* is almost exclusively encountered: the writer has examined only two rat-derived strains of other type: one *B. aertryck*, the other the Suipestifer strain of Kerrin (1928).

Pathogenicity of Salmonella Bacilli for Rodents under Experimental Conditions.

We are concerned here only with the broad facts, so far as they are known, regarding the differential pathogenicity of the types, leaving for later consideration the process and conditions of infection. If only by the toxicity of bacilli actually introduced, all laboratory rodents may be killed by parenteral injection of any Salmonella type, though the lower limits of commonly lethal dosage vary enormously with organism and animal. Intravenous and intraperitoneal injection are the most readily fatal modes of inoculation; subcutaneous injection is as a rule less regular in its result; still less certain, especially where small doses are employed, is peroral administration, and it is here that the most striking intertypal differences in pathogenicity are encountered. Of laboratory animals the mouse is the most readily and widely susceptible; the susceptibility of the guinea-pig is not vastly less: the rabbit is relatively refractory to perenteral infection; the rat to perenteral and parenteral infection, by the majority of types; but both rabbit and rat are markedly susceptible to particular organisms.

Salmonella Bacilli and the Destruction of Rodent Vermin.

Loeffler (1892[1 & 2]) at once grasped the idea that the bacillus, *B. typhi murium*, an Aertryck type organism, which had worked havoc among the laboratory mice at Greifswald might be turned to account against their wild congeners. Not only the house-mouse but also the field-mouse, *Arvicola arvalis*, was found susceptible, and over both signal successes were claimed in practice. But Loeffler's organism formed no panacea against the rodent plague: it proved powerless against the rat and *Mus agrarius*. As a result *B. typhi murium* and other murine strains of Aertryck serology have been largely superseded in interest and application by a series of Enteritidis type organisms of more polyvalent pathogenicity for myomorphs: Mereschowsky's (1894) bacillus from epidemic disease of *Spermophilus musicus*, Issatschenko's (1898, 1902) bacillus from rats and, among others, those three strains on which attention has mainly focussed—the organism of Danysz (1900) from *Arvicola*, 'Ratin', isolated by Neumann in 1903 from the urine of a child with cystitis, and 'Liverpool virus'. The first essential in the application of these various organisms is that they should cause a high percentage mortality among the animals actually consuming the infected bait; this end may be equally attained by the use of ordinary rat poisons: it is the presumption that the disease will be communicated from animal to animal by contact and cannibalism which, in the case of these so-called 'rat viruses', has fostered larger

hopes. Much labour has been expended in efforts to increase and to maintain the virulence of the strains, and in comparisons of their relative killing power under laboratory conditions. In experiment all these organisms have given a high mortality figure, often 100 per cent. for white mice, sometimes rather less for grey mice ; in the case of the rat reports on the efficiency of the Enteritidis type ' viruses ' have varied, the mortality figures given by feeding experiments ranging from less than 20 per cent. to over 90 per cent. : Steffenhagen (1911) gives an excellent summary of the laboratory experiments with regard to rat destruction. Bahr admits that some 10 to 20 per cent. of rats resist Ratin, but states (Bahr, 1918) that when the bacillary culture is supplemented with a preparation (Ratinin) of squills this immunity is broken down. In field practice results have been as discrepant as in the laboratory : Danysz and Issatschenko both claimed highly satisfactory results with their respective organisms, and Bahr (1909, 1918, 1923), basing his opinions on results obtained by the systematic use of Ratin in a large number of Danish provincial towns, and pleading with the conviction of experience, presents the ' Ratin system ' as the ready-to-hand remedy against the rat pest. Against these claims must, however, be measured the results of others who, working with one or other of these organisms, found that the rats either avoided the bait, merely migrated, or eating the bait did not disappear : in similar contrast with Loeffler's favourable results in the use of *B. typhi murium* for mouse extermination are those of various observers, including Messerschmidt (1921), who found the method of little or no avail.

Mouse-Feeding Experiments in the Differential Diagnosis of of *B. aertryck* (*B. breslaviensis*) and *B. paratyphosus B.*

The fact that orally administered to mice in large doses, *B. aertryck*, the Breslau bacillus, normally sets up lethal septicæmic infection, usually fatal within ten days, while *B. paratyphosus B* usually does not, has been utilized by the adherents of the Kiel doctrine for the practical differentiation of these two organisms.

Salmonella Infections of Swine.

By far the most frequent Salmonella invaders of the pig are, in their respective areas, the American and European Suipestifer types. To the former, as the Hog-cholera bacillus or *B. choleræ suis*, Salmon and Smith (1885) ascribed the prime role in the causation of hog cholera ; and the latter, its then undifferentiated congener, was accepted under the title of *B. suipestifer* (Kruse) as cause of the corresponding European disease of swine (swine fever or Schweinepest). Grave doubt was cast on this view by the fact that the alleged causal organisms were not always to be found in the affected swine and that experimental administration of pure cultures did not set up the symptoms of the disease ; doubt became certainty when Dorset and Schweinitz and Dorset, Bolton and MacBryde in America, and,

in Europe, Uhlenhuth, Hübener, Xylander and Bohtz (1908, 1909), v. Ostertag, Hutyra and others, demonstrated the virus ætiology of hog cholera and Schweinepest. What is the pathological significance of the organisms thus dethroned? As earlier stated there is evidence that, locally or generally, Salmonella bacilli, probably for the most part, *B. suipestifer*, are present, perhaps as saprophytes, in the intestines of a certain but variously estimated percentage of normal swine. In swine fever (hog cholera) the proportion of animals infected by these bacilli is greatly increased (20 to 100 per cent.). There seems good reason for belief that both types of *B. suipestifer* and various other Salmonella types play a definite, if secondary, role in the disease (swine fever); in particular in the causation of the characteristic button ulcers and other necrotic changes in the intestine.

Against the view that the various Salmonella forms encountered in the pig play a solely secondary part in its diseases a definite reaction has arisen. Outbreaks and sporadic cases of disease associated with *B. suipestifer* or related bacilli and in which the Schweinepest virus was apparently absent, have from time to time been reported, and the reports are being rapidly augmented; it is urged that there exists, quite apart from virus Schweinepest, a bacillary Schweinepest—a 'Paratyphus des Schweines'—and a specific bacillary disease of sucking-pigs—Ferkel-typhus. The issue was first raised by the observations of Glässer (1907, 1908, 1909) and Dammann and Stedefeder (1910) on outbreaks of Schweinepest-like disease among young pigs, in which no virus could be demonstrated. In most cases bacillary Schweinepest takes a chronic form with wasting and diphthero-necrotic changes in the gut; in the circulation the bacilli are usually scanty, though they may be obtained from the organs *post mortem*. Acute cases, however, occur, marked by vigorous bacillæmia, splenic enlargement, acute lymphadenitis and hæmorrhages. It would seem that very frequently 'primary' Salmonella infection depends on predisposing causes such as damp and cold conditions, and, according to Manninger (1925), diet deficient in calcium salts, in protein or in vitamin; it has also been suggested that *B. suipestifer* may acquire a virulence, hitherto lacking, by passing through the pig. Meyer and Boerner (1914) believed that *B. abortus equi*, while causing but trivial symptoms in the adult pig, had a definite action on the uterus and its fruit, inducing abortion. Pigs have been found unaffected in feeding tests with mouse typhoid bacilli and the various Enteritidis type rat-viruses.

Salmonella Infections of the Horse.

Most important of the equine Salmonelloses are infectious abortion of mares and certain maladies of the foal. The great majority of the cases are due to the organism first isolated by Kilbourne (1893) and Smith (1893) from aborting mares in the United States. From many names proposed, we have selected as title for the organism, that suggested by

Meyer and Boerner (1913)—*B. abortus equi*. In the mare infection is usually benign—a fever, abortion, lingering metritis—the foal is stillborn or diseased and little viable. Foals may suffer post-natal infection through the navel or *per os ;* in older animals there is often joint-localization of the disease. According to Lütje (1924) *B. abortus equi* (*paratyphus equi*) is responsible for 30 to 70 per cent. of equine abortions, and for at least 12, possibly 20, per cent. of the diseases of the foal after birth. Occasionally the organism sets up fatal septicæmic disease in adult horses. Such cases are usually solitary, but there are probably to be included the epidemics, reported by Combes (1918[1 & 2]), Moulin and Amichau (1918) and Amichau (1918), which during the War severely ravaged transport and artillery horses at Grenoble and Lyons ; it would seem that in these outbreaks several types of bacterial infection were concerned, and it is difficult to escape the conclusion that the organisms isolated, including those which we presume to have been *B. abortus equi*, were secondary invaders in a disease undiagnosed. In diagnosis of the disease considerable use has been made of agglutination tests performed with the blood-serum of the mare ; since the normal agglutinative titre of mare's blood often extends to a dilution of 300, readings, made with fresh serum, must exceed this titre to be significant ; it appears, however, that if the test-sera are heated for a short time at 56° C. before test their non-specific action is deleted, leaving the specific effect undisturbed. The organism is to be cultured from the fœtal tissues and blood, from the vaginal secretion and uterus of the mare, and, at autopsy on the latter, it has been found in the liver, spleen, and other organs.

Though it is alleged that carrier-stallions, discharging the organism in their semen, have been demonstrated, and though spread of infection during coïtus has been suspected, experimental evidence of the importance of seminal carriers is lacking (see Pröscholdt, 1924).

Though Lütje (1924) doubts the agency of other Salmonellas in these maladies of mare and foal, it is clear from the reports of Miessner (1925), Pfeiler and Heinrich and others that Breslau (Aertryck) bacilli are sometimes concerned. Hutyra and Marek (1922) ascribe to Graham, Reynolds and Hill observations on an outbreak of disease among horses due to Gärtner's bacillus. Kutscher and Meinicke (1906) report that Pfeiler found two horses severely affected by feeding with Loeffler's *B. typhi murium*, but that they found another unaffected by like administration of *B. paratyphosus B*.

Salmonella Infections of Sheep.

There are remarkably few records of Salmonella disease in sheep. One of the most interesting relates to the sheep epizootic at Überruhr (Bruns and Gasters, 1920) in which 160 sheep perished or were emergency slaughtered, with symptoms of enteritis and ' Paratyphus B ' (actually Aertryck) infection ; sold for food the flesh of these animals caused some

2,000 cases of acute food-poisoning. Another important outbreak, briefly referred to by Jordan (1925), affecting 30,000 sheep in Colorado, was likewise due to *B. aertryck*. German bacteriologists (Schermer and Ehrlich, 1921 ; Stephan and Geiger, 1922 ; Miessner and Baars, 1927) have drawn attention to the activity of a special Salmonella type, *B. abortus ovis*, in the disorders of lambing. The organism is to be isolated from all the organs of the affected animals, from the uterine discharge of aborting ewes and from the fœtal tissues ; well-marked specific agglutinins develop, titres of 400 upwards being accepted as diagnostic. The infected ewe may exhibit no obvious symptoms of disease.

Salmonella Infections of Cattle.

B. enteritidis is the most frequent Salmonella invader of cattle : one of the main causes of calf diarrhœa (dysentery), it is still more frequent in the pulmonary and less localized septicæmia maladies of the calf ; is responsible for most of the relatively rare Salmonella infections of adult cattle and for certain cases of bovine abortion (3 per cent., Lütje). Cases of Gärtner infection in adult cattle, to knowledge of which Lütje (1926) makes a valuable contribution, are for the most part scattered, but epidemics, of which Lehr (1927) and Lütje give examples, occasionally occur. Second in importance to Gärtner infections are those due to *B. aertryck :* most of the bovine ' Paratyphus B ' strains of Continental authors (Titze and Weichel, 1910 ; Zschiecke, 1918 ; Christiansen, 1914) were certainly or probably of this type, and there must be added the ' calf typhus ' strains of TenBroeck (1920) and the calf organism of Mackie and Bowen (1919). Uhlenhuth and Seiffert (1926) and Shibata (1927) allege isolation of *B. paratyphi B hominis* from a calf. The only other Salmonella type known from bovines is the rare form *B. morbificans bovis* obtained by Basenau (1893) from a cow.

Salmonella Infections of Carnivores.

Information with regard to carnivores is fragmentary. Instances are recorded in which dogs and cats seem to have been involved in outbreaks of human food-poisoning ; one such has come within the writer's experience.

Salmonella Infections of Apes and Monkeys.

The only two records—apart from a possible instance of typhoid infection—of spontaneous Salmonella disease in these animals concern European *B. suipestifer* : Bernhardt (1913), probably to be credited, described as identical in their peculiarity two strains, one isolated from a monkey, the other from human food-poisoning and now known to be of the said Suipestifer type ; acceptance of the Simian strain, not now available for study, as *B. suipestifer* is encouraged by Schütze's (1920, ' G ' strain) isolation of this organism from a monkey.

Salmonella Infections of Birds.

The types mainly concerned in the Salmonelloses of birds are *B. aertryck* (Breslau b), *B. sanguinarium* and *B. pullorum*. In its role as avian parasite and under the name *B. psittacosis*, *B. aertryck* first came under notice as cause of infectious enteritis in parrots (Nocard, 1893). Knowledge of the so-called Paratyphus infections of the fowl, turkey, goose and duck is in somewhat uncertain state. The strains isolated in America from ' keel ' in ducklings under the title *B. anatum* (Rettger and Scoville, 1920) seem to be for the most part *B. aertryck*. The course and morbid anatomy of these Salmonelloses of parrots, song-birds, pigeons and poultry conform very much to one pattern. With limply drooping wings and disordered plumage, the bird assumes a dejected appearance ; it becomes rapidly emaciated ; diarrhœa is usually marked and respiratory trouble common.

There remain for discussion two sharply defined diseases of the chick and fowl which are of considerable economic importance. First of these is *bacillary white diarrhœa of chicks*, elucidation of which is due to the labours of Rettger and his associates (Rettger and Harvey, 1908 ; Rettger, 1909). This disease occurs as a rule during the first week or ten days after hatching : the symptoms are loss of appetite, diarrhœa with whitish stools, ruffled coat, drooping wings and exhaustion ; the post-mortem features are not striking—a pale slime-filled intestine, a pale or blotched liver, sometimes showing focal necroses and petechial hæmorrhages. The incidence of the disease in affected broods and the resultant mortality are usually very high ; the usual mortality figure is in the region of 70 to 90 per cent. The causal organism, *B. pullorum*, abounds in heart-blood, internal organs and intestine. An important feature is that many of the female survivors of these outbreaks become ovarian carriers of the organism and act as reservoirs of infection : the ova themselves bear the taint and from them develop diseased chicks, which, in their short lives, convey the disease to their healthy companions. Of more important contributions to knowledge of ovarian carriers of *B. pullorum*, their frequency, and their activities are those of Rettger and Stoneburn, Gage (1911), Rettger (1914), Doyle (1925) and Beller (1926). Jones (1913[1]), Knight (1924) and others have applied agglutination tests, made with the blood of the fowl, to detection of carriers and selection of an infection-free breeding stock : positive reaction at or below serum concentration, 1/33 or 1/50, is accepted as significant of infection ; Doyle (1925) regards agglutination at 1/25 as diagnostic. Active infection of adult stock by *B. pullorum* is relatively rare and usually accounts only for sporadic deaths ; occasionally, however, it causes epidemic disease of grown birds (Jones, 1913[2]).

The second important Salmonellosis of the fowl passes under the name *fowl typhoid* (Hühnertyphus). At successive investigations the causal organism has been named *B. gallinarum* (Klein, 1889[1 & 2]), *B. sanguinarium* (Moore, 1896) and *B. typhi gallinarum alkalifaciens* (Pfeiler and

Rehse). Unlike bacillary white diarrhœa of chicks fowl typhoid is seldom seen in the first days after hatching ; it may occur from the end of the first week onwards through life. The facies of the disease is that typical of avian Salmonelloses ; on an average 80 to 90 per cent. of birds in the affected flocks are attacked and of these, 50 to 90 per cent. succumb.

Experimental Study of Salmonella Infection and Toxicity.

Infection.

The Course of Infection.

Knowledge of the process of *perenteral infection* is mainly due to the labours of Max Müller (1912[1]) who, working with Enteritidis, Aertryck and other strains, and with the mouse as test animal, concluded that in the case of organisms of ordinary virulence the sequence of events was as follows : invasion of the lymphatic elements, buccopharyngeal and intestinal, of the alimentary wall ; spread to the nodes of mesentery, neck, axilla and popliteal region ; thence by lymphatic channels to spleen and liver ; thence, usually between the second and third day, into the blood-stream, setting up transient bacillæmia ; retreat to spleen, liver and lymph nodes ; multiplication therein through 2, 3 or 4 days ; reinvasion, this time progressive, of the blood-stream, generalization of infection. Müller considered that only where virulence and toxicity of infection were intense did direct invasion of the blood-stream occur, but that, in such case, the primary septicæmia might develop forthwith into the main attack. The bacilli were discovered in muscle, bile and urine only after septicæmia had become established. The special permeability of the lymphatic system was illustrated in striking manner by the case of strains of low virulence : organisms which had long lost power to set up septicæmic infection and others, such as *B. typhosus*, normally avirulent by ingestion were found capable of entering and colonizing the lymphatic system, persisting for considerable periods in the lymph nodes.

Müller's story of perenteral infection has been confirmed in most essentials by later work with the mouse and other laboratory rodents (Lange and Yoshioka, 1924 ; Elkeles, 1926 ; Ørskov, Jensen and Kobayashi, 1928). The role of the intestinal lymphatic apparatus as main portal of infection and the early ephemeral septicæmia—which latter, though not evident in the protocols of Webster (1923[2&3]), seems fully confirmed—are points of special interest. Ørskov, Jensen and Kobayashi suggest that the bacilli, having gained the peritoneal cavity, pass into the circulation via the thoracic duct to be filtered off in liver and spleen, so determining infection of these organs : a more plausible interpretation than that of Müller. Various descriptions, relating almost exclusively to Aertryck infection of the mouse, of the fortunes of the bacilli in the intestine itself vary in detail. Most observers (M. Müller, 1912[1] ; Lange and Yoshioka, 1924 ; Barnewitz, 1924 ; Hage, 1925) agree that the ingested bacilli show little immediate tendency to multiply in the

alimentary canal, usually undergo reduction in number, and often remain inconspicuous or undemonstrable in gut contents and droppings during incubation of the disease ; with onset of septicæmia and symptoms their number usually increases rapidly, and they may overwhelm the normal coliform or other flora of the intestine ; occasionally, however, the bacilli remain undemonstrable in the fæces through the entire course of septicæmic disease. The observations of Ørskov, Jensen and Kobayashi (1928), relating the rapid increase of bacilli in gut and stools, not directly to the septicæmic state, but to infection of the bile, bring explanation of these irregularities. Müller's statement regarding invasion of the lymphatic system by organisms of low virulence has been confirmed by Adler (1925) for *B. typhosus*, by Elkeles (1926), the writer, and others for *B. paratyphosus B*.

Parenteral infection. Salmonella bacilli introduced into the pleural and peritoneal cavities of susceptible animals show, as Webster (1922[1]) confirms, an initial lag-phase, lasting a few hours, during which their number decreases ; then follows steady increase, maintained till death of the host. In the case of subcutaneous injection initial spread is largely by way of lymphatic channels. Meyer, Neilson and Fusier (1921) compared the behaviour of *B. typhosus*, *B. paratyphosus A* and *B. paratyphosus B* (including *B. aertryck*) when injected intravenously into rabbits. Typhoid and paratyphoid A bacilli were, with rapidity varying with the size of the dose, filtered off from the circulation in liver, spleen, lungs and bone-marrow and there vigorously destroyed ; quite early, sometimes in a few minutes, the bacilli appeared in the bile, presumably by way of the capillaries of the liver, and so descended to the intestine, their continuance in this last depending on persisting infection of the bile-tract ; appearance of the organism in the urine was for some days deferred and was sometimes found associated with lesions in the kidney. Paratyphoid B bacilli were likewise removed from the blood-stream and concentrated in the viscera ; eventually, however, in contrast to events in the case of *B. typhosus*, the process was reversed and the bacilli multiplying rapidly, re-established septicæmia and overwhelmed the host ; there was some evidence that gut infection might arise by escape of bacilli through the intestinal wall as well as through the bile-tract. Ørskov, Jensen and Kobayashi found that Breslau bacilli (1,000 bacilli) introduced into the circulation of the mouse are filtered off in less than three hours ; there follows a varying period in which the blood is sterile ; then septicæmia recurs.

Chronic Infections.

In laboratory rodents surviving natural or experimental Salmonella infection the bacilli in many, probably the majority of cases, persist for weeks or months in the organs, particularly spleen, liver, gall-bladder and lymph-nodes ; sometimes they are continuously or intermittently shed in fæces, urine or both : among recent records are those of Amoss (1922[2]), Topley (1926) and Knorr (1926) regarding mice.

Gall-bladder infections. The bulk of experimental work on Salmonella infections of the rodent gall-bladder has been effected with *B. typhosus*: to the article on this organism the reader is referred; here we note only conclusions generally applicable and certain studies based on other members of the group. Apart from direct operative inoculation of the gall-bladder there are three routes by which the organ might conceivably be invaded. Most modern opinion agrees that ascent of the organism from the intestine by way of the common bile-duct—a process contemplated by Lorey, Kliewe (1922) and others—does not occur. There remain as alternatives, or as joint possibilities, direct infection of the gall-bladder from the blood (hæmatogenous infection) and secondary infection brought by the bile-stream from the liver (hæmato-hepatogenous infection). It has been found that Salmonella bacilli, usually in test typhoid bacilli, intravenously injected into rabbits and guinea-pigs after ligation of the cystic duct may, often in a few minutes, appear in the hepatic bile, while the cystic bile-remains sterile. Meyer, Neilson and Fusier (1921), however, concluded that there may be early invasion of the cystic bile through the gall-bladder wall itself, and were inclined to believe that descending hæmato-hepatogenous infection of the gall-bladder sets up in the rabbit only superficial catarrhal cholecystitis and determines only a temporary carrier condition; that the persistent carrier state arises as result of bacterial embolism in the gall-bladder wall with consequent necrosis and development of deep and infected lesions extending to the mucous surface. We have, however, isolated *B. aertryck* from the bile of a rabbit in which a gall-bladder was entirely lacking, eight weeks after the animal had been perorally inoculated with that organism. The most important studies involving organisms other than *B. typhosus* are those of Fränkel and Much (1911), who succeeded in inducing cholecystitis in guinea-pigs by peroral and perenteral administration of an organism from perityphlitic pus; of Wagner and Emmerich (1917), who set up a fæcal carrier condition enduring several weeks in guinea-pigs by direct inoculation of the gall-bladder with A and B paratyphoid bacilli; and of Arai (1923), who, using immunized rabbits, inoculated Paratyphus B bacilli intravenously, into the gall-bladder or into bone-marrow, and concluded that the last-named method determined cholecystitis of a type most nearly approaching that seen in human gall-bladder infections.

Arai also investigated the sister problem *renal excretion of paratyphoid bacilli* and concluded that in the rabbit the organism passes readily through the undamaged kidney to appear in the urine; indeed it was found difficult to induce infective localization in the organ by chemical injury or mechanical trauma; localization, however, followed ligation of the ureter.

Factors Influencing Infection.

Leaving for later attention the specific influence of acquired immunity and accepting the platitude that the result of exposure to infection is a function of the susceptibility of the proffered host and the virulence of

the parasite we consider first *variation in individual susceptibility* of the animal. Within wide limits of dosage equal administration of virulent Salmonella bacilli to each of a series of animals does not, as a rule, result in death of all : examination of the survivors shows that some develop agglutinins, while some do not ; some killed after an interval show lesions attributable to the organism, some harbour the organism itself, in others no evidence of infection present or past is discernible. This irregularity in the fate of individuals is usually ' explained ' as due to difference in their susceptibility, though the query may be raised, as it has been by Topley, as to how far irregularity depends on innate or intrinsic differences in power to resist infection, how far on unavoidable fortuities of experiment. In some cases survival may be favoured by immunity acquired by earlier infection, a possibility never to be entirely excluded in work with laboratory rodents.

Virulence.

Recent Work on Salmonella Virulence and its Fluctuations.

The variability of Salmonella virulence in both senses, admitted by most workers, has been denied by Webster (1923[5&6]) : studying strains of *B. enteritidis* and *B. aertryck* tested in doses ranging from several million to a few hundred bacilli, and repeatedly subjected to mouse passage, this observer concluded that the virulence of each strain was fixed and was unweakened by laboratory culture and unexhalted by mouse passage. The justice of this conclusion has been called in question by Topley (1926), from whose laboratory Lockhart (1926) reports experiments, likewise effected with Aertryck strains and mice, in one of which, it is claimed, the organism emerged from the tenth mouse passage with killing power definitely increased ; Lockhart concludes that while the effect of animal passage is quite uncertain it may provoke an appreciable increase of virulence. Claim to have raised the virulence of the hog-cholera bacillus by serial passage through rabbits was made by TenBroeck (1917). There remains the valuable study of Bahr (1925, 1927) on the virulence of an organism of the Enteritidis type—presumably the ' Ratin ' strain. Twenty-five races of this strain were tested perorally on groups of rats (4 to 20 rats per group) : the observed mortalities in these groups varied from 100 to 0 per cent., and the differences noted in the case of some of the larger groups leave little doubt that marked differences in virulence were indeed present. Bahr impresses, what is indeed the lesson of virulence studies throughout, the vastly differing effects of mouse and rat passage ; that while an indefinite series of mouse-to-mouse passages (either by direct feeding with infected organs or with intervening cultivation) may be maintained, rat passage sooner or later leads to loss of virulence—sometimes in the first few passages, sometimes not till the 100th to 200th passage. Bahr further considers the relative virulence—as determined by the use of 2,828 rats—of 596 cultures of his strain in relation to the time to death of the animals from which they were isolated. He concludes

that the highest percentage of virulent cultures, as reckoned either by a 50 or 70 per cent. mortality standard, are to be isolated from animals perishing between the fourth and sixth days.

Relation of Serological Variation to Virulence.

Topley and Ayrton (1924[2]) found that the specific and non-specific phases of *B. aertryck* show similar perenteral virulence for mice ; they were at first led to conclude that the specific phase, alleged to occur with special frequency in the spleens of survivors, was seldom, as was the non-specific phase, excreted in the fæces ; this thesis was, however, abandoned. It remains a possibility that change of phase within the host —a change known to occur *in vitro* under the influence of homologous serum—may nullify in part the reaction of the animal to an original infection. Between the smooth organism and its rough variant a remarkable difference in infectivity and virulence exists : the virulence of the rough organism is regularly low (Grote, 1913 ; Schütze—personal communication, 1921 ; Topley and Ayrton, 1923, 1924[1]—*B. aertryck ;* Goyle, 1926[1]—*B. enteritidis ;* White, 1926 and unpublished observations—*B. suipestifer* and various Salmonellas ; Arkwright, 1927[1]—*B. paratyphosus A ;* Webster and Burn, 1927[1, 2, 3 & 4]—*B. enteritidis*). Peroral inoculation of laboratory animals, susceptible to the smooth form, with large doses of rough culture seldom, if ever, decides septicæmic infection by the inoculated form though occasionally the bacilli attain the lymph-nodes and liver ; very rarely reversion to smoothness with resultant infection seems to occur in mice. Subcutaneous injection of considerable, intraperitoneal injection of small or moderate, doses is, as a rule, hardly more effective ; large intraperitoneal doses may in some cases at least—the guinea-pig and mouse and rough Aertryck and Enteritidis strains—set up a fulminating septicæmia ; large intravenous doses often determine a protracted or chronic type of disease. This low virulence of rough variants does not appear, at least in all cases, to be due solely to immediate destruction of the bacilli : we have isolated rough typhoid and Aertryck bacilli from the circulation and heart-blood of rabbits 7 to 10 days after intravenous injection of rough cultures.

So far as we are able to judge from their writings, Topley (1926) and Lockhart (1926) are not inclined to regard smoothness and roughness as covering the real epidemiological problem of fluctuating virulence ; they would seem to consider with greater interest the lesser changes which they have reported, and which, in that they concerned morphologically smooth races only, they apparently deem of distinct nature. It must, however, be argued that roughening—serologically, chemically and physically—is far more a process than an event. It seems to us probable that this process in its essentials—reduction of the specific carbohydrate and those internal changes on which this reduction must depend—may be far advanced before it finds expression in cultural roughness. In the present state of knowledge it remains a tenable hypothesis that variation

in virulence, even within the range of the smooth colony, is correlated with variation in degree of smoothness. Though it would be rash to suggest that the soluble specific carbohydrate component itself decides virulence and its variations there is reason to regard it as an important factor in the decision : *in vivo* as *in vitro* this substance must act in favour of the freedom of the individual units, and so facilitate their active and passive dispersal ; diffused it must at least tend to neutralize, and thus render ineffective, those factors in the host fluids which threaten its precipitation on the bacteria themselves with consequent agglutination and immobilization of the latter.

Along the lines of the aggressin experiments of Bail and those recently performed by Felton and Bailey in the case of the soluble specific carbohydrate of the pneumococcus, the writer has made a few experiments to determine the possible influence of an excess of the soluble specific substances of Aertryck, Paratyphosus B, and Enteritidis strains on the course of infection by the homologous organism. The number of experiments performed is too small to warrant any final conclusion ; further, it must be admitted that intravenously injected the solutions of soluble specific substance employed were definitely, if but slightly, toxic—a feature, we now believe, not to be attributed to the soluble specific carbohydrate itself ; nevertheless, for what it is worth, the observation may be recorded that in every case there was a difference—sometimes that of death and survival, sometimes only in the day of death—between the fate of the test animals receiving (subcutaneously) both culture and soluble specific substance and those receiving culture alone ; at the doses employed the soluble specific substance injected alone and subcutaneously caused no symptoms. Here was added in excess a reagent already present in the bacilli, and, taken as a whole, the experiments suggest a resulting intensification of the infective process ; they, therefore, suggest that variation in the degree of development of this element in living cultures may be reflected in the killing power of the races.

In view of the importance which, in this connection, we are inclined to ascribe to smoothness and roughness, and of the fluctuations in virulence alleged to occur, in both directions, as a result of animal passage, there arises the question of the *influence of animal passage on smooth and rough races.* There is no doubt that rough bacilli, rarely encountered in acute generalized infection, are apt to occur in localized and chronic disease. From the livers and spleens of trapped and laboratory rats the writer has isolated rough *B. enteritidis ;* from chronic infection of guinea-pigs rough variants of the Reading type and *B. enteritidis ;* from Aertryck-immune rabbits subsequently injected with the homologous smooth organism rough variants of the latter. In the case of man, the writer (White, 1925) has obtained rough *B. paratyphosus B* from the fæces of a carrier and rough *B. aertryck* from the pus of an ischio-rectal abscess : there stand, too, the reports of Gildemeister (1916, *B. paratyphosus B*, carrier stools) and Wagner (1920, *B. paratyphosus A*, abscess). In this

connection may be recalled the fact that roughening is often to be induced *in vitro* by the action of smooth antiserum. There seem to be no records of isolation of rough variants (apart from actual inoculation of such) from mice : indeed Webster and Pritchett (1927) in an extensive search through 13 samples of Salmonella-infected mice from various sources report entirely negative findings on this point. We are tempted to suggest that there is a connection between the absence or infrequency of rough variants in the mouse and the failure of mouse passage to reduce virulence, and conversely a connection between the apparently not infrequent occurrence of these variants in the rat and the established influence of rat passage in reducing virulence. It seems that active parasitism may in some cases stimulate return to smoothness. Two clear cases of such reversion, in which any confusion with latent infection of the experimental animal could be excluded, have come within the writer's experience : in one was noted reversion of a rough Newport strain during guinea-pig passage ; in the other, accompanied by the rough infecting form, smooth *B. typhosus* was obtained from the gall-bladder of a rabbit dying on the twenty-eighth day after intravenous injection of rough typhoid bacilli : both reverting races were serologically proved. There is, therefore, some indication that those conditions which have been held responsible for variation in virulence are those calculated to cause change from smoothness to roughness and vice versa.

Toxicity.

That agar and broth cultures of Salmonella bacilli, saline autolysates of the former and filtrates of the latter possess poisonous properties was determined by the pioneer workers on the group and many others.

Toxic Broth Culture Filtrates.

It has been the usual observation that filtrates of ageing and autolysed broth cultures (5 to 14 or more days old) are of much higher toxicity than those obtainable after 24 to 48 hours' growth ; the rise of toxicity revealed by successive samplings corresponds with that period during which, the maximal density of growth having been attained, the count of variable bacilli in the culture is decreasing rapidly—that is from about the fourth or fifth day onwards (Smith and TenBroeck, 1915 ; Branham, 1925). Note must, however, be made of the contrary finding of Ecker (1917) and Ecker and Richardson (1925), who, demonstrating definite toxicity at the eleventh hour of growth, obtained their most marked animal reactions with filtrates of cultures 24 to 48 hours old : nevertheless, most of those who have reported negative or ill-defined results have restricted attention to young cultures. Yoshioka (1923) places the moment of maximal toxicity about the third week. In most studies the medium has been a meat-extract or meat infusion peptone broth, the Witte brand being by general assent the peptone of choice. The peptone content of the medium is of some importance in that peptones may themselves be toxic : media containing 2 per cent. of Witte peptone have

been reported satisfactory. The addition of small quantities of glucose (0·1 to 0·2 per cent.) has been regarded as favourable (Smith and TenBroeck, 1915; Bahr and Dyssegaard, 1927). Litch and Meyer (1921) urge importance of an alkaline medium (pH 8·4); Bahr and Dyssegaard accepted the range pH 7·5 to 8; Yoshioka (1923) found initial acidity desirable in the instance of *B. typhosus* but in that of *B. paratyphosus B* apparently obtained best results with an alkaline medium; Ecker (1917) and Ecker and Richardson would seem to regard alkalinity as inessential. Cultures have as a rule been incubated at 37° C.; Bahr and Dyssegaard consider 30° C. more favourable. Often the grown culture covered with toluol has been allowed to continue autolysis at room or incubator temperature (Cathcart, 1906). Most workers admit wide variation in potence of filtrates obtained, under seemingly similar circumstances, from different strains of a type or from a single strain.

Thermostability of the Salmonella 'toxins'. Notwithstanding certain earlier statements to the contrary the toxic properties of Salmonella culture and broth-filtrate alike are not appreciably affected when the latter are heated for an hour or more at 70 to 80° C. or for 10 to 15 minutes at 100° C.; in some cases at least, steam pressure of 10 pounds is resisted, though we have found the toxicity of agar-grown bacilli to be destroyed in half an hour at 15 pounds steam pressure.

Toxic Action by Parenteral Injection.

In demonstrating the toxicity of cultures and culture filtrates, the rabbit has been favourite test animal: for detailed description of the reaction of the rabbit to Salmonella 'toxin' intravenously injected the reader is referred to the paper of Smith and TenBroeck (1915) who write more particularly of the 'toxins' of the fowl typhoid bacillus: many other descriptions, tallying therewith and relating to other Salmonella types, will be found in the literature cited in the introductory note. In all or almost all cases a definite period, the so-called incubation period, about 40 to 50 minutes in duration, elapses between intravenous (or intraperitoneal) injection of 'toxin' and onset of acute symptoms: though Yoshioka states that large doses of his potent filtrates cause death with violent symptoms in a few minutes, most workers have found the length of the incubation period largely independent of the size of the injected dose. Onset of acute symptoms is heralded by agitation; then more or less rapidly the animal becomes prostrated: with toneless muscles, limbs helplessly extended and head thrown back, it lies panting; the body temperature falls; there is usually copious discharge, first of fæcal pellets and urine, then of fluid fæces. The symptom picture bears a marked resemblance to anaphylactic shock. Provided death does not supervene the acute and demonstrative attack passes into a condition of drowsiness, almost typhoid-like, from which the animal may be roused, but into which it at once relapses. Death where it occurs is convulsive; it occurs as a rule within 24 hours, often between the second and fifth

hour, but may be deferred for 2, 3 or 4 days. Animals which recover show loss of weight, which is slow of correction, and often persisting paresis of the hind limbs. In other laboratory animals intravenously or intraperitoneally injected there are the same features of prostration and diarrhœa ; respiratory difficulty seldom takes the same demonstrative form as in the rabbit ; in dogs, cats and monkeys vomiting may occur. The doses of broth culture filtrate found fatal to mice by intraperitoneal injection have been in the order of 0·1 to 0·5 c.cm. ; those lethal to guinea-pigs by the same route and to rabbits intravenously have for the most part ranged between 0·5 and 5 c.cm. ; filtrates fatal to rabbits in 0·02 to 0·05 c.cm. amount have been sometimes obtained (Messerschmidt, 1912 ; Yoshioka, 1923). It is general experience that, making allowance for size, the rabbit is the most sensitive of laboratory animals. Subcutaneous injections are as a rule without effect.

The Question of Enteral Toxicity.

Certain observers (Gärtner, 1888 ; v. Ermengem, 1892 ; Tiberti, 1908 ; Uhlenhuth and Hübener, 1913) have reported illness, sometimes associated with posterior paresis, hæmorrhagic enteritis, and even resulting in death, in animals receiving killed cultures of Gärtner and ' Paratyphus ' bacilli *per os :* others, notably Bahr and Dyssegaard (1927), have administered enormous quantities of killed culture or culture filtrates by the mouth to mice, rats, guinea-pigs, rabbits, dogs, pigs and monkeys without eliciting sign or symptom of disease. Savage and White (1925[1]), seeking to reproduce certain phenomena of human food-poisoning in laboratory animals, were baffled by the lack of response shown by the latter to large peroral doses of *B. aertryck* and *B. enteritidis* killed at 60 or 100° C. ; only in one instance did diarrhœa result, and in only 2 or 3 rabbits of many tested were slight signs of prostration observed : it was, however, noted that massive doses of heat-killed agar-grown culture, particularly of culture heated at 100° C., might, in a few hours, and usually without causing marked indisposition, set up gastritis of varying degree : in a few instances, coinciding with those in which slight prostration occurred, the condition was severe and comparable with that seen in the stomach of human food-poisoning ; in these cases the stomach was congested and œdematous with points and patches of hæmorrhage over the swollen rugæ of the fundus ; in the majority, however, this measure of inflammation was not seen and in many the reaction was trivial. Menten (1926) apparently reports the same reaction of the rabbit stomach to concentrated Salmonella ' toxins ' but little confirmation has come from other sources. It has been the experience of those engaged in study of immunization by the enteral route that mice succumb to large doses of killed Enteritidis or Aertryck culture given *per os.* In this connection the recent paper of Branham, Robey and Day (1929) is of interest : these workers report that, though autolysed, boiled or autoclaved suspensions of washed *B. enteritidis, B. aertryck* and *B. paratyphosus B* given *per os*

to mice have little effect, boiled broth cultures and broth culture filtrates, especially young cultures and their filtrates, delivered into the stomach in 1 c.cm. amount, cause death, usually between the seventh and fourteenth day, in 40 to 100 per cent. and 40 per cent. of mice respectively ; they found the potence of broth cultures to be actually increased by autoclaving, but to fall with filtration ; they doubt whether the poisonous principle concerned is that active in human food-poisoning.

Intertypal Differences in Toxicity.

The writer's experiments and those of others indicate that sterile filtrates and autolysates from all or almost all the common Salmonella types intravenously injected into laboratory animals produce approximately the same effect : there is certainly nothing here to aid explanation of the intertypal differences which mark the Salmonella intoxications of man. Speaking next of injection intravenously or intraperitoneally of large doses of bacilli, living, killed at 60° C., at 100° C., or otherwise prepared, of different Salmonella types, the writer must in large part rely on his own experience : his experiments have concerned *B. aertryck*, *B. enteritidis*, *B. paratyphosus B* and *B. typhosus* and have been made with rabbits, mice and rats.

Rabbits vary widely in the degree of their reaction to like injections of agar-grown Salmonella bacilli : to formulate a strict rule as to their behaviour is impossible. Though, when living Aertryck or Enteritidis bacilli, in young culture and in doses of 1 to $1\frac{1}{2}$ agar-slant culture, are intravenously injected into rabbits, death usually occurs within 24 hours, there is seldom very severe prostration at the end of the first hour ; in the case of *B. paratyphosus B* the early reaction tends to be still less marked ; in that of *B. typhosus*, on the other hand, the effect of such an injection is usually fulminating and fully comparable to that of a toxic broth culture filtrate. Where cultures killed at 60° C. are injected in like amount the violence of the intoxication set up by *B. typhosus* is hardly modified, while the effect of the other types named is often quite trivial. It seems, however, that the toxic action of the food-poisoning and paratyphoid B types may be increased by heating the suspensions at 100° C.

The contrast in the behaviour of living and heat-killed typhoid and paratyphoid B bacilli has been noted by many, including Meyer, Neilson and Fusier (1921), who group *B. paratyphosus A* with the former in this matter. In the case of the mouse intravenously injected with bacilli killed at 60° C., *B. enteritidis* has, in our experiments, consistently exhibited the most severe early toxicity, that of *B. aertryck* and *B. paratyphosus B* being definitely less ; *B. typhosus* has tended to cause late death after several days of apparent well-being. In the white rat bacilli of all types examined, killed at 60° C. and intravenously injected, have, after a short incubation period (40 to 50 minutes), set up acute intoxication (prostration and diarrhœa), often ending fatally with convulsions in a few hours ; in

the case of young rats (100 to 120 gm.) half a normal loopful of agar growth is usually lethal ; *B. typhosus* seems to be, dose for dose, more violent in action than *B. aertryck*, *B. paratyphosus B* and *B. enteritidis*. Savage and White (1925[1]) believed that they could demonstrate a difference in irritant action on the rabbit stomach between Aertryck and Enteriditis bacilli on the one hand and paratyphoid B bacilli on the other by giving large doses of boiled agar-grown culture *per os ;* in the case of the food-poisoning types inflammation was usually definite ; in that of the para-typhoid type negligible. While the writer is confident that some difference of action does exist, he doubts the truth of the explanation earlier advanced —namely, that the food-poisoning types possess a gastro-irritant poison lacking in *B paratyphosus B.*

Toxicity of Rough Variants.

We have frequently found that, in contrast to the corresponding smooth products, filtrates and supernatants of ageing broth cultures of rough bacilli possess very little toxicity, and Goyle (1926[1]) made the same observation in the case of aqueous autolysates of *B. enteritidis*. In comparative tests with living and heat-killed (60° C.) agar-grown bacilli a corresponding difference is at least in certain cases patent. A striking instance is, in our experience, afforded where the said variants of *B. typhosus* are intravenously injected into rabbits ; whereas one agar-slant culture of the smooth organism constitutes a probably fatal dose, two—even three—slant cultures of the rough form are commonly tolerated ; though, sometimes, where the living organism is used, setting up a chronic emaciating disease. It seems, however, from our experiments that when rough Salmonella cultures are treated with alcohol before injection their toxicity may equal, or even excel, that of smooth *B. typhosus*. We feel forced to the conclusion that rough organisms possess much the same potential toxicity as the smooth form but that in them for some reason the toxic substances are not to the same degree liberated and brought into effect.

General Nature and Origin of the Salmonella ' Toxins '.

Three possibilities have been suggested. One is that the Salmonella poisons are products of the action of the organism on the nutrient medium ; split products of enzyme action. That such products may contribute to the toxic effect is difficult to disprove ; that they are inessential to typical display of Salmonella toxicity is demonstrated by the fact that the toxic property is shown, at least in parenteral injection, by fresh or alcohol-treated cultures of washed bacilli and by filtrates of culture in simple ' synthetic ' media such as that of Braun and Cahn Bronner (1921), Branham (1925), Ecker and Richardson (1925), Ecker and Rimington (1927). Another possibility is that the poisonous substances are comparable to the so-called ' true toxins '—those of *C. diphtheriæ*, *B. botulinus* and *B. tetani :* against this interpretation stands the opinion of most workers that significant appearance of Salmonella poisons in the culture medium depends on death and disruption of the bacilli ; then there is the

matter of resistance to heat ; the comparatively low titre of their toxicity ; their disproportionably low efficiency by the subcutaneous route. The third suggestion, that the Salmonella poisons are in the nature of endotoxins, presents two subordinate possibilities : one is that the endotoxins exist as such in the living organism ; the other that they are break-down products of its decay. This view, without decision between the alternatives which it includes, is that usually accepted ; though in the present state of knowledge the term endotoxin conveys little enough in definition.

Concentration of the Toxic Substances.

Menten (1926) found that the toxic and glycæmia-inducing substances in filtrates of broth cultures of *B. aertryck* and *B. enteritidis* could be precipitated with acetic acid. Ecker and Rimington (1927), dealing with filtrates of 24-hour cultures of '*B. paratyphosus B*' (actually *B. aertryck*) in synthetic broth, state that they were able to throw down the toxic fraction with lead acetate, and, by decomposition of the lead components, to obtain a potent product, containing 0·3 to 0·4 per cent. of nitrogen, giving negative protein reactions but strong indications of a carbohydrate component—with which last they were cautiously inclined to associate the toxic property. The observations of Ecker and Rimington might tend to raise suspicion that the toxic principle of the Salmonella is related to the specific serologically active carbohydrate of the smooth bacillary body : since solutions of the latter, potent in precipitation tests but devoid of toxicity, may be obtained, the possibility may be dismissed.

Some Problems of Immunity to Salmonella Infection.

Attempts to Immunize the Mouse against Mouse Typhoid Infection.

Early workers (Loeffler, 1906 ; Wolf, 1908 ; Yoshida, 1909) demonstrated the possibility and the difficulties of immunization. Among recent experimenters, Webster (1922[1]), employing a heat-killed vaccine, was able, with a double or triple subcutaneous inoculation, to protect 8 out of 40 mice tested by intraperitoneal infection, and 21 out of 40 tested by oral infection. Immunization *per os* did not give such good results, unless a living vaccine was used, in which case mortality during treatment was correspondingly great. Ornstein (1922), Lange and Yoshioka (1924) and Neufeld (1924), mostly using chloroform-killed vaccines, lost many mice under treatment and achieved but slight protection. Topley, Wilson and Lewis (1925) fed mice for one month with living Aertryck bacilli ; 50 per cent. died during this time ; 32 survivors were tested with an intraperitoneal injection of 200,000 organisms ; the mortality was 12·5 per cent. as compared with 76·7 per cent. among the controls. Kurokawa (1926) obtained similar protection after similar losses during vaccination by rubbing living organisms into the scarified skin. Ørskov, Jensen and Kobayashi (1928) compared carbolized and formolized vaccines ; the latter were less toxic ; both achieved equal degrees of immunity—a single

intravenous or subcutaneous inoculation of 100 million bacteria protected 22 per cent. against a lethal oral test dose. Springut (1927) lost many animals during immunization with a large number of killed vaccine inoculations and achieved a 90 per cent. immunity. Ibrahim and Schütze (1928) lost only 2 mice out of 75 during immunization with three doses and protected 50 per cent. of the animals.

Solid immunity to *B. aertryck* is obviously so far unobtainable without a heavy death-roll during treatment.

Immunization Experiments with other Laboratory Rodents.

Rabbits and guinea-pigs are easily immunized with living Aertryck or Paratyphoid B vaccines; given 0·01 c.cm. living broth culture subcutaneously, followed after two or more weeks by 0·01 c.cm. injected intravenously, rabbits are immune to an intravenous test dose of 1 c.cm. Rabbits immunized with 1,000, 2,000 and 4,000 million heat-killed bacilli of almost any type intravenously at intervals of seven days can resist 0·25 c.cm. of living broth culture of the homologous organism. Kutscher and Meinicke (1906), Howell and Schultz (1922), Nichols and Stimmel (1923) immunized guinea-pigs similarly against Aertryck and Paratyphoid B. Ornstein (1922), using chloroform-killed vaccine, did not succeed.

The attempts at immunization of rabbits with *B. suipestifer* have met with little success. Living vaccines cannot be used owing to the extreme virulence of this organism for that animal; killed vaccines are ineffective. Wassermann, Ostertag and Citron (1906) discovered that rabbits and guinea-pigs surviving infection with *B. typhi murium* were resistant to *B. suipestifer*. TenBroeck (1918), Schütze (1921) and White (1925, 1926) found that good immunity to *B. suipestifer* may be set up by inoculation with living Aertryck vaccine. Johnson (1921) even achieved fair immunity with killed Aertryck vaccine. These findings led to a number of experiments by TenBroeck (1920[2]), Schütze (1921) and White (1925, 1926) in which cross-immunity between many of the Salmonellas was shown to be possible when living vaccines were used. The significance of these results is uncertain and does not appear to depend on the presence of antigen common to the types in question. Perhaps the phenomenon of depression immunity (Lange, 1921) is involved.

It may be mentioned here that Herzog and Schiff (1922) reported an immunity in typhoid convalescents to a *B. enteritidis* food-poisoning outbreak, while Perry and Tidy (1919), on the other hand, decided that 'T.A.B.' vaccination had no influence on the Newport epidemic they studied.

Antitoxin Immunity.

The more important contributions are those of Franchetti (1908), Ecker (1917), Ecker and Richardson (1925), Branham (1925) and Branham and Humphreys (1927). Sera definitely possessing low antitoxic powers

have been obtained by repeated inoculation of rabbits with culture material. Cross-protection by antisera of the different types has not been attempted.

Immunization of Animals as a Practical Measure.

Vaccine and serum prophylaxis and therapy have been applied with varying success to the case of the Salmonella infections of food animals. Considerations of space forbid any proper survey of veterinary practice in this field ; the diseases on which attention has been mainly riveted are the septicæmic and dysenteric Salmonelloses of the calf, equine abortion, Ferkeltyphus and fowl typhoid. In the case of the so-called Paratyphus maladies of the calf the serum of immunized horses has been used both for preventive and for curative purposes, usually by subcutaneous injection of 15 c.cm. or larger quantities. Some measure of success appears to have attended attempts to reduce the losses of mare-abortion by vaccination of the mare before, or at an early stage in pregnancy, either with killed cultures of *B. abortus equi* or with an extract of the bacilli (Parabortin). The communications of Miessner and Berge (1917), Pfeiler (1919), Ruppert and Porcel (1924), and Gminder (1920) may be mentioned. Pfeiler and Kohlstock (1914) took up the question of active immunization of sucking-pigs against Ferkeltyphus (Glässer-Voldagsen) infection by means of killed cultures, and claimed success ; similar measures have been adopted in bacillary Schweinepest (Schweineparatyphus). Good results have also been reported in the case of poultry stocks vaccinated with killed cultures of *B. sanguinarium* against infection with that organism. Several workers faced with severe losses among their laboratory animals have undertaken systematic vaccination of their stocks. Both Lynch (1922) and Howell and Schultz (1922) considered that the subcutaneous injections of killed bacilli which they administered were responsible for the abatement of the mouse and guinea-pig epizootics with which they respectively dealt, and occasioned in the one case by *B. enteritidis*, in the other by *B. aertryck*.

Normal 'Antibodies' to the Salmonella.

Questions of natural immunity, of variation in resistance, racial and individual, standing complementary to those of infection have of necessity been dealt with in writing of the latter. Here it remains to consider very briefly such physical display of resistance as is discernible in the normal host, leaving out of count the widely operative phenomenon of cellular reaction, phagocytosis.

Normal Agglutination.

The blood sera of the larger domesticated animals have, at least in a high proportion of cases, a definite agglutinative action on Salmonella bacilli. The reader is referred to the observations of M. Müller (1912[2]), Savage (1918), and Kinloch, Smith and Taylor (1926). In the case of cattle the titre, usually negligible in the calf, may rise to 800 ; values of 100 to 200 are common. Though irregularities in the reaction of different

bacillary suspensions are met with, we doubt if any marked intertypal differences of reaction exist. Breinl (1920) seeking to analyse the agglutinative action of normal ox-serum on typhoid, A and B paratyphoid and Gärtner bacilli concluded that as in the case of immunized animals, distinct finely clumping and coarsely clumping agglutinins exist, though in his experiments only *B. enteritidis* consistently showed both types of reaction. Breinl favours the view that the agglutinins of infection and vaccination arise by hypertrophy of rudiments, pre-existing and already qualitatively defined, in the normal animal. Savage and Kinloch, Smith and Taylor take the view that the Salmonella agglutinins of the healthy ox and other food animals are probably the aftermath of an earlier infection : such an interpretation would, however, seem to suggest just that broadcast occurrence of Salmonella bacilli in these animals which the bacteriological findings of Savage's investigation appear to negate. In the rabbit agglutinative action on the smooth Salmonella seldom attains a titre above 10 ; in mice the value is practically zero ; in rats a titre of 25 or 50 for *B. enteritidis* may usually be accepted as indicating past or present infection. While the writer cannot claim to have fully elucidated the matter he has little doubt that the normal agglutinative action of ox- and horse-serum on smooth Salmonella bacilli is of the same general nature as that studied in the case of rough variants (see p. 106) : that the serum factors concerned, save in occasional instances, bear any relation to specific agglutinins—are agglutinins stimulated by transient infection or agglutinins in embryo—he doubts profoundly. A point brought out by Müller is that this agglutinative action of the sera of food animals is not accompanied by any similar activity on the part of the muscle juices : in infected and vaccinated animals, on the other hand, these juices have considerable potency in this respect.

Bactericidal Action of Normal Serum and Lymph.

In examination of normal bactericidal action *B. typhosus* has been the usual object of study : information regarding the A and B paratyphoid types is far less developed, regarding the remaining types, almost negligible. It has been the general experience that *B. paratyphosus B* is far less susceptible to the sera of mammalian species than is *B. typhosus ;* so far as the facts are known *B. paratyphosus A* follows the example of the latter. In the rabbit, among laboratory animals, the contrast in this respect is particularly striking : Meyer Neilson and Fusier (1921) state that rabbit sera of which 1 c.cm. might destroy 5 million typhoid bacilli were usually found incapable of disposing of so few as 15 paratyphoid B bacilli. In some experiments by the writer, 2 of 11 normal rabbit sera were found to have definite bactericidal action on paratyphoid bacilli : action on *B. aertryck* though never approximating to that on *B. typhosus* was usually more marked than on *B. paratyphosus B.* Gonzenbach and Uemura (1916), confirming the contrast in the susceptibilities of *B. typhosus* and *B. paratyphosus B* to the sera of various animal species, upheld earlier

statements regarding the higher bactericidal potence of plasma: this latter circumstance they ascribed to availability of substances otherwise lost by absorption in the clot. From studies based on *B. typhosus* regarding the bactericidal value of inactivated blood-serum and lymph — the literature of which may be gleaned from the papers of Pettersson (1924) and Ikegami (1925)—we single out a series of experiments by Ikegami dealing with the action of fresh and inactivated dog lymph on A and B paratyphoid bacilli. Bactericidal action of the fresh lymph on *B. paratyphosus A* was as marked as that on *B. typhosus*, and on both these organisms the inactivated fluid still exerted a bactericidal or growth-inhibiting influence; growth of *B. paratyphosus B*, retarded by fresh lymph, was unaffected by that inactivated at 56° C. Ikegami states that, while in the dog the 'thermostable' bactericidal substances occur both in blood and lymph, they appear in the rabbit to be restricted to the latter. It will be recalled that G. Seiffert (1917), writing of typhoid and mouse typhoid infections, pointed to the possession of these 'thermostable' factors as the brand of susceptibility to attack, to their absence as the hall-mark of immunity: however, Wöhlisch (1919), comparing in this regard the serum reactions of typhoid and paratyphoid convalescents, anti-typhoid vaccinated subjects and normal persons, encountered complete irregularity of behaviour.

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CHAPTER III. THE DYSENTERY GROUP OF BACILLI.

By A. D. Gardner (Oxford University) and L. Dudgeon (St. Thomas's Hospital, London) with Sections by W. Bulloch (London Hospital) and R. A. O'Brien (Wellcome Laboratories).

History.

By W. Bulloch, London Hospital.

The history of *Bacillus dysenteriæ* really begins with the paper of Kiyoshi Shiga (1898) of Kitasato's Institute in Tokio. At Kitasato's suggestion he tried to identify the ætiological agent of acute dysentery by applying serological methods to bacteria obtained by culture from the dysenteric stools. In 34 out of 36 cases Shiga found in the dejecta a bacillus, markedly resembling the typhoid bacillus, which agglutinated with the patients' sera. Shiga described this bacillus as slowly motile, and Gram-negative. It produced no liquefaction of gelatin, did not clot milk, did not ferment glucose, produced no indole, and the growth on potato was practically invisible. Living cultures produced pathogenic effects, but not clinical dysentery in guinea-pigs, puppies and kittens. The bacillus was agglutinated by the serum of dysenteric patients but not by other sera, normal or pathological. Shiga inoculated himself subcutaneously with a killed culture of the bacillus. A rather severe local and general action followed and in ten days his serum showed the same agglutinating effect as that of patients who were suffering from the disease. Considering all these properties, Shiga was of opinion that the bacillus was the cause of dysentery. His earliest account was supplemented by others which he published in 1901, 1902 and 1908.

Two years later, W. Kruse (1900), at that time Professor in Bonn, gave an account of bacilli which he had isolated from dysenteric cases in Laar (Westphalia). These bacilli, with minor differences, appeared to be the same as Shiga had found in Japan, and Shiga agreed with this opinion. Continuing his work, Kruse (1901) found other bacilli, especially in the asylum form of the disease, which were not the same. Kruse spoke of them as ' Pseudo-dysenteric ' bacilli. In the course of an investigation (1899) of tropical diseases among American troops in the Philippine Islands, S. Flexner (1900) found in dysenteric cases a bacillus apparently identical with that of Shiga. Strong and Musgrave (1900) in Manila also isolated a similar organism. It was pathogenic and produced dysentery in an Indian under sentence of death. Flexner (1901) made a comparative study of the various strains of bacilli isolated from dysentery, and concluded that there was no doubt of their identity. An important paper of Martini and Lentz (1902) revealed the fact, however, that agglutinating dysenteric serum from human dysenteric cases is not a trustworthy reagent for bacterial differentiation. With an agglutinating serum prepared by inoculating a goat with a pure culture of the true Shiga

strain, they found that different reputed strains of dysentery bacilli resolved themselves into two groups, of which one contained the bacilli of Shiga and Kruse, whereas an entirely different group included the Flexner Manila strain, the bacillus of Strong and Musgrave, and the 'pseudo-dysenteric' strains of Kruse. Searching for other means of differentiating the two groups Lentz (1902) found that in litmus-coloured media containing mannitol the Shiga group produced no change, whereas the Flexner (Manila), Strong, and pseudo-dysenteric bacilli all produced an acid reaction. All subsequent work has confirmed the statements of Lentz. A soluble toxin produced by the Kruse strain was found by C. Todd (1903, 1904). Considerable interest was excited in a particular bacillus isolated by Hiss and Russell (1903) from the stools of a fatal case of diarrhœa in a child. This bacillus which they designated as 'Y'—the reason is not apparent—was found to be a mannitol fermenter, and in this respect differed from the bacilli of Shiga and Kruse. Hiss (1904–5) published a classification of dysentery bacilli based on fermentation and agglutination tests. He distinguished four groups represented by the Shiga, Flexner, Strong and Y bacilli respectively. For a time this classification was widely accepted, but it became apparent that the mannitol fermenters comprise a very heterogeneous collection which differ serologically, as was first clearly made out by Kruse, Rittershaus, Kemp and Metz (1907), who distinguished a large number of groups. This was roughly speaking the state of knowledge in 1914. In all the theatres of the war there were enormous numbers of cases of dysentery, and the bacteriological aspect of the subject was studied on a corresponding scale. The great variability of so called 'Flexner' strains was emphasized everywhere, and much was done by serological analysis to define the groups. In England, Andrewes and Inman (1919) were important supporters of the doctrines of Kruse and his co-workers. The general result of bacteriological studies of dysentery bacilli during and since the war has shown that the question is more complicated than was believed, and much requires to be done before the modern doctrines can be regarded as stable.

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The Group as a Whole.

By A. D. Gardner.

Definition and Description.

The dysentery bacilli are small aerobic (facultative anaerobic) non-motile, Gram-negative rods, which have no spores, capsules nor pigment, and do not liquefy gelatin. They ferment without gas-production glucose and a varying number of other carbohydrates and alcohols. The chief members of the group are the infecting agents in bacillary dysentery, a disease characterized by the frequent passage of stools containing blood and mucus, accompanied by straining and tenesmus, and only distinguishable from amœbic dysentery by microscopic and bacteriological examination. It is the lack of motility and of gas-production, combined with distinctive serological reactions that serves to distinguish the dysentery bacilli from other members of the typhoid-coli group.

It is a matter of great difficulty to define the limits of the group. Our present knowledge is far from complete, and our classification can therefore only be provisional. Medical bacteriology desires a sharp distinction to be made between the organisms that cause dysentery and those that do not, while pure bacteriology aims at a biological arrangement of all the related bacilli according to their most fundamental characters, of which pathogenicity is only one. In this chapter a compromise is adopted by including in the dysentery group various organisms that are not, in the medical sense, true dysentery bacilli, but are clearly very closely related to them. The classification in Bergey's Manual of Determinative Bacteriology, which is largely based on that of the American Committee of Bacteriologists, does not seem likely to establish itself firmly, and is therefore not followed in this chapter. The various bacilli of the dysentery group are presented in Bergey's Manual as species of the Genus *Eberthella* of the Tribe *Bactereæ* of the Family *Bacteriaceæ ;* Order *Eubacteriales* and Class *Schizomycetes*.

In Table I are given the names and the chief distinctive characters of the more important members of the group, of which the first three and probably the fourth, *B. dysenteriæ* (Schmitz), are the dysentery bacilli proper. The classification of the mannitol-fermenters into ' Flexner ', ' Y ', and ' Strong ' has been rejected for reasons given in the section on the ' Flexner group '.

Morphology and Staining Reactions.

There is nothing in the morphology and staining reactions of the dysentery bacilli to distinguish them from other non-motile members of the typhoid-coli group. Growth and multiplication are of the normal type. Just as in many other species of bacilli, the rejuvenating growth from old cultures often contains branched or ' Y ' forms (Hata, 1908), which reproduce the normal bacilli by triple budding or ' three-point multiplication ' (Gardner, 1925). Colony-variation into ' smooth ', ' rough '

TABLE I.

The Dysentery Group. Distinctive Characters.

Name of Variety	*B. dysenteriæ* (Shiga)	*B. dysenteriæ* (Flexner group)	*B. dysenteriæ* (Sonne)	*B. dysenteriæ* (Schmitz)	*B. alkalescens*	Dysentery-like bacilli (miscellaneous)
Ætiological connection with dysentery.	Positive	Positive	Positive	Almost certainly positive	Almost certainly negative	Negative
Serological (agglutinative) characters.	Distinctive, uniform	Distinctive, diverse, but related	Distinctive, probably uniform	Distinctive, probably uniform	Distinctive, ? diverse	Diverse and unrelated
Lactose	O	O	A (early or late)	O	O	O (? sometimes late A)
Glucose	A	A	A	A	A	A
Mannitol	O	A	A	O	A	A
Indole	O	+*	O*	+	+	Variable
Milk	Slight changes A–N	Slight changes A–N	Increasing strong acidity and often coagulation	Slight changes A–N	Slight A then strong Alk.	Variable

A = Acid. Alk. = Alkaline. N = Neutral. A–N = Primary acidity, followed by neutrality. O = No reaction.
+ = Positive reaction. * Many authors consider the reaction inconstant.

(Arkwright, 1920) and even ' mucoid ' (Fletcher, 1920) is not uncommon, particularly in old strains. Old colonies on agar of the Flexner and Sonne groups, and ageing growths on many sugar-media tend to produce daughter-colonies, that is to say, knobs of fresh growth not spreading beyond the limits of the mother-colony. The daughter-colonies often have increased fermenting powers.

Motility. The statement that the dysentery bacilli are non-motile is based on an overwhelming majority of the written records, on the general consensus of bacteriological opinion, and on the writer's own experience. Nevertheless, it has not passed completely unchallenged at any period since the discovery of the first bacillus of the group by Shiga in 1898. Both Shiga and Flexner (1900[1 & 2]) described the dysentery bacillus as motile, and it was left to Kruse (1900) to lead opinion in the opposite direction. From this time on the bacteriological world became more and more convinced that the dysentery bacillus possesses no true motility. Nevertheless, Vedder and Duval (1902) claimed to have stained flagella, and Birt and Eckersley (1905) believed themselves to have demonstrated motility and flagella in dysentery strains of various origin. By means of a special technique Duval and Bassett (1904) seemed to have induced motility in originally immobile cultures, and about the same time further records of motile dysentery bacilli were published by Dunn (1904) and Torrey (1905). A more restricted motility in the form of active rotation or simple oscillation without progression has been asserted by Ruffer and Willmore (1909) in a group of ' Y ' strains isolated in Constantinople, and by Remlinger and Dumas (1915) in similar bacilli from Argonne, which were certainly true dysentery bacilli. Vincent and Muratet (1917) accept an extremely transient oscillation of freshly isolated strains as characteristic, and quote Sir Patrick Manson's authority that both Shiga and Flexner are flagellated bacilli; although there is no evidence that Manson's opinion was based on his own observations. The ' unusually active Brownian movement ' described in most text books seems to be a somewhat meaningless compromise. It is likely that at least some of the observations just recorded may be explained by the practise of making hanging drops for the observation of motility, and films for flagella staining, with tap water containing motile bacilli, or with contaminated saline solution.

Differentiation by Cultural Methods.

The growth of the various bacilli of the group in all ordinary culture media, apart from those mentioned in Table I is, with the following exception, fairly uniform and does not lend itself to differentiation.

The Sonne type is exceptional in that the habitual growth of most strains on agar and in broth approximates more to the ' rough ' than to the ' smooth ' type of growth, i.e. on agar these bacilli form rather flat and slightly granular colonies, as contrasted with the normally circular and evenly translucent colonies of the other varieties. In broth they flocculate spontaneously. The writer has found simple stab-culture, in

agar a useful differential test, for Sonne strains usually spread from the centre to the periphery of the upper surface in 2 or 3 days, whereas the other dysentery bacilli, in their normal ' smooth ' phase, grow at first only on a restricted area around the entry-point of the needle. Flexner-group strains are said to produce on an average denser growths than Shiga. For distinguishing dysentery bacilli from certain organisms that may be confused with them Sonne (1915) recommends potato. On this medium dysentery-group bacilli grow in a delicate, almost invisible film in 24 to 48 hours, whereas the imitating bacilli produce a thick yellow growth.

Odour. Fresh cultures of the four ' true ' dysentery types, Shiga, Flexner, Sonne and Schmitz all possess a characteristic spermatic odour. There is no record of a similar odour having been observed in other (atypical) members of the group. Winter (1912) found the odour to vary with the strain, some being very strong and others odourless. Acidity of the culture-medium reduces, alkalinity favours the odour production.

Differentiation by Biochemical Reactions.

Milk.

It has been stated from time to time that the reactions of the dysentery group in milk are too inconstant to be used for differentiation (e.g. Sonne, 1915), and it is true that reports have often been conflicting, particularly concerning the behaviour of the Sonne type (q.v., p. 244). Nevertheless, the great majority of bacteriologists find it a valuable differential medium, and there is no doubt that the reactions given in Table I are true of the average specimen of fresh and reasonably clean milk.

Sonne and others prefer litmus whey, which they allege to have a more constant composition than milk. It is stated that in this medium all dysentery bacilli produce a transient acidity, followed by a return to neutrality, which is permanent in the case of Shiga and the Flexner group, but soon gives way to a second and lasting acidity with cultures of the Sonne type. It was shown, however, by Thjøtta (1917) that these changes are by no means constant, either with different strains of one type in a single batch of medium, or with one strain when tested in different batches of medium.

Carbohydrates, &c.

With the exceptions of lactose, glucose and mannitol none of the substances commonly used as fermentable reagents for the differentiation of the dysentery bacilli have stood the test of time (see Flexner group).

Peptone water has usually been found a suitable medium for fermentation tests, but Andrewes and Inman (1919) recommend Winter's (1912) ovomucoid medium for the detection of minor degrees of acidification (a 4·5 per cent. solution of dry hens egg-white in cold water is steamed for an hour and filtered ; a half per cent. each of NaCl and of the carbohydrate are then added). Hiss's serum-water medium gives good results, but is more difficult to make, and does not seem to possess any clear advantages. When peptone is used, it is important to employ a

sufficient concentration of the carbohydrate. Coincident with acid production from the carbohydrate there is a production of alkali from the peptone ; and it is important not to allow the latter to mask the former. One per cent. of carbohydrate is the minimum for safety. Some authors recommend 2 per cent. or more.

Lactose. There is evidence that the Shiga bacillus attacks lactose to a slight degree, but the acid produced is usually too weak to show (Winter, 1912). One may certainly sometimes see transient faint acidity in lactose media inoculated with either Shiga or Flexner bacilli ; but there is no danger of confusing it with the intense though late acid reaction caused by the Sonne type. Thomson and Mackie (1917) and Ledingham (1918) claim to have seen lactose-fermenting variants of Flexner group bacilli. According to Lentz (1913), the Y bacillus of Hiss gives pinkish colonies on litmus-lactose-agar, which indicates a slight acid-production from the lactose ; not, however, sufficient to cause any confusion.

Mannitol. This substance provides a fundamental test for distinguishing the Flexner-Sonne group from Shiga's and Schmitz's bacilli. Nevertheless, even here, the constancy of the reaction is not absolute. Failure of Flexner strains to acidify mannitol just after isolation has been reported by Park and Carey (1903), Shiga (1908) and Bauch (1918). This phenomenon, however, is usually not due to a real failure of fermentation, but to a temporary excessive alkali production, which diminishes to normal as the strains are subcultured (Lentz, 1913). Cunningham and King (1917–18) made similar observations, and also found a number of strains that on first isolation showed a delay of some days in mannitol-fermentation. With 1 per cent. solutions, some fresh cultures may produce only a transient acidity, and then revert to an alkaline reaction. In these cases 2 per cent. of mannitol gives permanent acidity. Finally there is a bacillus isolated by Denier and Huet (1913) in Indo-China, the bacillus ' Saigon ', which was described as having the cultural and serological characters of a Flexner strain except that it did not ferment mannitol. Examination by the writer in 1928 of a culture of this bacillus, obtained from the Institut Pasteur in Paris, proved it to have acquired the power of rapid acidification of mannitol—serologically it showed more affinity to Sonne's bacillus than to any of the stock (V to Z) Flexner types. Again, the loss of the power of mannitol-fermentation in an old strain has been noted by Calalb (1925). Agglutinability was lost at the same time. Fletcher and Jepps (1924) isolated non-mannitol-fermenting dysentery bacilli in Malay and found that they could be classed serologically as a mixed XY type of the Flexner group. Fletcher's bacilli were watched for several months without any change being observed, but there is no certainty that after a longer period the power of mannitol-fermentation would not have become manifest in these strains, as it has done in the Saigon bacillus.

Gas-production. The practically universal belief that dysentery bacilli do not produce gas in their fermentation of carbohydrates is well-grounded on innumerable observations, but, nevertheless, some rare exceptions have

been recorded. A certain number of careful observers have seen a slight and transitory gas-production in glucose by freshly isolated strains of Shiga, and in glucose and mannitol by the Flexner group (Rajchmann and Western, 1917). Goldzieher (1919), who quotes a number of earlier and similar observations, saw a little gas produced in glucose by 2 out of 28 Shiga strains and 27 of 66 of the Flexner group. A similar phenomenon has been recorded for the Sonne type.

Shiga strains of this kind might easily be confused with Morgan's bacillus, but would be distinguishable by their lack of indole production, non-motility and serological reactions. Flexner bacilli possessing a similar peculiarity could only be identified by the last two of these tests.

Acid Agglutination.

Michaelis (1915, 1917) believed it possible to distinguish true dysentery bacilli from non-pathogenic ' dysenteroids ' by the failure of the former to clump under the influence of acetic acid in suitable concentrations. Andrewes and Inman (1919) found the reaction sufficiently constant in the case of Shiga and Flexner bacilli, but it is to be noted that they exclude from the true dysentery bacilli the Sonne and Schmitz types, both of which are agglutinated by acid. In the hands of Murray (1918) and Dudgeon and others (1919) the reaction proved too inconstant for practical use ; and with this view we are in agreement.

Differentiation by Serological Reactions.

Agglutination and Absorption of Agglutinins.

Agglutination with specific agglutinating sera prepared from rabbits distinguishes the dysentery group swiftly and surely from its near neighbours, and the main classes of the group from each other (Martini and Lentz, 1902). When these sera and dead formalinized broth suspensions of the organisms are used, the coagglutination of related groups of bacilli is negligible. On the contrary the serological overlapping of groups or races is emphasized by the use of living cultures suspended in saline. Within the various subdivisions of the group the degrees of serological similarity of the races are very different. Complete in the case of Shiga, and probably also in Sonne and Schmitz, it changes to a wide heterogeneity in the Flexner group, which comprises an indefinite number of serological races that can be grouped around a limited number of type strains.

The last group of Table I is a miscellany of dysentery-like bacilli without serological coherence or pathological significance.

Human sera from dysenteric subjects are poorly suited for differentiation. The serum from a case of Shiga infection may agglutinate Flexner strains as highly or even more highly than Shiga bacilli, whereas the converse, i.e. agglutination of Shiga by Flexner sera seldom, if ever, occurs. The Sonne type is not agglutinated by Shiga or Flexner sera from infected human beings or from immunized animals. A similar absence of coagglutination is the rule for the Schmitz bacillus.

Horses are bad animals for the production of highly specific agglutinating sera, since their blood contains a great deal of normal agglutinin for the Flexner group. Goats are, for a similar reason, inferior to rabbits. Our information is, however, very incomplete as to the character of the serological reactions resulting from the immunization of a large range of animals against the various dysentery bacilli. It is, indeed, possible that the serological classification obtained with rabbit sera might not hold good with the sera of all other animals.

The chief difficulties of agglutination-work with this group are caused by the variability of the bacilli. All varieties are subject to fluctuations of agglutinability, which may be associated with definite changes of antigenic structure, as in the case of the ' rough ' modification which flocculates spontaneously in salt solutions and is distinct from the corresponding ' smooth ' modification in both its agglutination and its absorption reactions (Arkwright, 1920). Complete or partial temporary inagglutinability in specific serum is by no means a rare phenomenon in the group (see Practical Diagnosis of the different bacilli). Wide variations of sensitiveness to specific agglutinating serum occur both in different races of one serological group and in the same race at different times. For instance, the agglutinability of a race of Flexner ' V ' that had been in use for years at the Standards Laboratory at Oxford suddenly increased fivefold without any morphological modification. Furthermore, Flexner suspensions that are hypersensitive to specific serum are equally so to normal human serum, a circumstance which profoundly affects the diagnostic limits of normal agglutination (Gardner, 1921). Finally, the susceptibility of a suspension to coagglutination by heterologous sera is closely correlated with its sensitivity to normal rabbit serum (Murray, 1918). With such facts before us we can hardly doubt the wisdom of eliminating these pitfalls by making use, wherever possible, of standardized suspensions of known and constant agglutinability, such as those prepared according to Dreyer's method (Gardner, 1918).

For the preliminary serological identification of a bacillus that gives the cultural reactions of the dysentery group Murray (1918) recommends that if the agglutination reaction with any type-serum be positive to a quarter or more of the titre of the serum, the bacillus be provisionally considered as serologically of that type.

The absorption of agglutinins may be of value in the typing of dysentery bacilli in difficult cases, or when the preparation of sera with the strains under examination is impossible. But the writer agrees with Murray (1918) that practically all the desired information as to the relationship of any two strains, e.g. of a new strain to a stock strain, is obtainable without absorption tests by careful titrations of the sera made with both strains by means of standardized suspensions of each. If the ratio of the degrees of agglutination of the two suspensions is the same (within the limit of experimental error) with the two sera, the strains may be taken as serologically identical.

The absorption test can be applied to the determination of the infecting agent in dysentery when the patients serum agglutinates one bacillus, e.g. Shiga, to a certain degree, and another, e.g. a Flexner strain, to an equal or higher degree. If all the agglutinins for both bacilli can be removed by saturation with a Shiga culture, it may be concluded that the Flexner strain was merely coagglutinated. If, however, there is a strong residuum of Flexner agglutination, the probability is that we are dealing either with a mixed infection, or with a Shiga dysentery in an individual who has been infected with the Flexner type in the past. Within the Flexner group partial absorption of heterologous agglutinins is the rule. The Sonne and Schmitz types are absorptively distinct from each other and from the Shiga and Flexner groups.

Complement Fixation.

This reaction has given valuable information about the serological constitution of the group (Haendel, 1908; Lunz, 1911; Schroeter and Gutjahr, 1911; Thjøtta, 1917). Dopter (1905) applied it in a rather crude manner, and believed it to establish the fundamental unity of Shiga and the Flexner bacilli. But all the other workers mentioned found a quantitative distinction between the groups, and there is no doubt that this is the truth, even though the reaction tends to reveal relationships rather than distinctions.

Here again rabbit-sera have been found to give the most clear-cut results. With asses' serum Haendel was unable to effect a reliable grouping.

Although Thjøtta detected differences of fixation within the Flexner group, he was not able to classify these bacilli as clearly as by agglutination. He had, however, no difficulty in distinguishing Shiga's bacillus from that group, and his Group III (Sonne) from both Shiga and Flexner. It is by no means uncommon to find *B. coli* strains that fix complement with dysenteric sera, human or animal. Thjøtta found this frequently with Group III (Sonne) sera, but he saw no fixation of the other group sera, either by *B. coli* or by *B. paratyphosus B.* *B. typhosus*, on the other hand, reacted to a fair degree with Shiga and Flexner sera, but not with those of the Sonne type. The reaction did not indicate any serological relationship between Sonne and the Shiga and Flexner groups.

We thus see a tendency for Sonne (the late lactose-fermenter) to range itself towards the *B. coli* side of the Typhoid-coli group, while Shiga and Flexner approximate more to *B. typhosus*. As regards the technique of the test: the most satisfactory antigens are 24-hours agar suspensions in saline, heated to 60° C. for 1 hour. Thjøtta found these definitely superior to solutions of bacterial substance in antiformin. They are also the best material for immunizing animals.

In the case of the Flexner group, various observers have been troubled by spontaneous fixation by the suspensions, which must therefore be used in a dilution great enough to remove this danger.

Bacteriolysis.

The same serological grouping can be established by means of bacteriolytic tests (see Vol. IX) as by agglutination and complement fixation. In the early days the Shiga and Flexner types had been found distinguishable by the reaction *in vivo*, and this was confirmed *in vitro* by Kruse, Rittershaus, *et al.*, in 1907. In a very thorough investigation of the subject Thjøtta (1917) gave a conclusive demonstration of these differences, showing that the Shiga bacillus, the Flexner group and the Sonne type gave distinctive reactions, but that the various races of the Flexner group could not be distinguished readily from each other by this test. Flexner bacilli were lysed by Shiga serum up to about an eighth of the titre, but the converse did not occur. A certain proportion of dysentery races were found to be altogether refractory to bacteriolysis. In contrast with complement fixation, this reaction showed no relationship between the dysentery bacilli and *B. typhosus*.

Precipitation (or Flocculation).

Anti-Shiga serum contains precipitin for both Shiga and Flexner races, though it acts more strongly on the former than the latter (Coyne and Auché, 1907). In a series of tests of dysenteric human sera with filtered cultures of Shiga and Flexner bacilli, Lancelin and Rideau (1918) found precipitins with regularity, but were unable to determine the type of infecting bacillus, because the precipitins were often equally strong for both. It is, nevertheless, probable that with a more quantitative technique a group-difference would emerge.

Bacteriotropic and Opsonic Actions.

The sera of animals injected with suspensions of Shiga or Flexner bacilli develop the property of intensifying phagocytosis, even after heating to 60° C. to destroy opsonins. There is a certain amount of cross-action on heterologous species within the group ; but its degree depends on the animal used. Asses' serum gives a strong, rabbits a weak cross-effect (Haendel, 1908). Part of the beneficial action of antidysenteric serum may be due to its bacteriotropic property. Dysentery antisera have also an opsonic action on the homologous bacillus, and some group-effect. The same property can be shown in normal adult human serum, and even in new-born babies. Dick (1911) raised the opsonic titre of normal individuals 7 or 12 fold by two subcutaneous injections of mixed Shiga and Flexner vaccines. On Flexner group bacilli Shiga antiserum has a stronger opsonic action than normal serum (Auché, 1908).

Pathogenic and Epidemiological Characters of the Group.

Types of Infection in Man.

It is generally admitted that, on an average, the Shiga bacillus produces a more severe dysentery and has a greater tendency to epidemic spread than the Flexner group or either of the remaining types. For instance,

75 per cent. of the severe cases of dysentery in the Salonika force during the war were caused by Shiga, and a similar proportion of the mild cases by the Flexner group, with an occasional Schmitz infection (Dudgeon and others, 1919; see also Lancelin and Rideau, 1918). Nervous symptoms, prostration, and, if the disease become chronic, permanent disablement are more frequently found in Shiga infections (Lentz, 1913). Carriers of Shiga bacilli are usually invalids; carriers of Flexner may enjoy apparently normal health.

The dysentery produced by the Sonne and Schmitz bacilli is seldom if ever as severe as the worse Shiga epidemics. Fraser, Kinloch and Smith (1926) found that 'in the great majority of cases of Sonne dysentery the symptoms are those of an irregular subacute diarrhœa with green stools containing mucus'. In fact, the relationship of Shiga to the other bacilli of the group has a certain resemblance to that of *B. typhosus* to the paratyphoid bacilli, a consideration which led Kruse to make a clinical division into (1) true dysentery of a primarily epidemic nature, and (2) a mainly sporadic pseudo-dysentery; and to erect a bacteriological classification on this basis. Kruse's terms, *B. dysenteriæ* for Shiga, and *Bacilli pseudodysenteriæ* for the Flexner-Sonne-Schmitz groups, have, however, never taken root outside the German-speaking world; for it has been reported from time to time from various countries that Flexner infections and epidemics may attain a virulence equal to or greater than that of Shiga (van Hoof, 1925; Hoshi, 1925). In some countries where dysentery is endemic, such as Norway and the Malay Peninsula, Shiga infections are the exception, and yet the severity of the disease and the frequency of epidemics may reach a high level (Thjøtta, 1917; Fletcher and Jepps, 1924). Finally, although the Sonne and Schmitz type are more commonly found in the sporadic than the epidemic form, undoubted epidemics caused by each have been recorded (Fyfe, 1927; Schmitz, 1918).

We believe, therefore, that we are justified in rejecting Kruse's classification and in adopting the generic name *B. dysenteriæ* for all the members of our group that have a proved ætiological connection with clinical dysentery, whether of a severe or of a mild type.

The summer diarrhœa of infants (epidemic or follicular enteritis) and other forms of diarrhœa. The summer diarrhœa of infants is neither clinically nor bacteriologically an entity. When caused by bacilli of the dysentery group it normally takes the form of an ileo-colitis and approximates to the classical picture of dysentery. When, however, dysentery bacilli cannot be found, the symptoms are more suggestive of a gastro-enteritis (Nabarro, 1923). In America, the dysentery bacilli, chiefly of the Flexner group, are the usual causal agent, and the diarrhœa is of the dysenteric type (Duval and Bassett, 1904; Wollstein, 1903; Torrey, 1905; Davison, 1922). In England, although sporadic cases of true dysentery in infants occur not uncommonly, the big outbreaks of diarrhœa in hot summers, with gastro-enteritic symptoms, are not due to dysentery bacilli (see Morgan's bacillus). In France, Auché and Campana (1905)

frequently found Shiga or Flexner bacilli in the dysentery of infants. German writers have commonly reported Flexner bacilli, or the Samonella group.

The Sonne type has lately been found with increasing frequency ; as a late lactose fermenter it has doubtless often been missed in the past (Hilgers, 1920 ; Davison, 1922 ; Webster and Williams, 1925).

In adults, diarrhœa without dysenteric symptoms may be associated with, and presumably caused by, true dysentery bacilli. In the Medical History of the War (Pathology) it is stated that ' In epidemic periods, and especially in the autumnal season, it has been possible to demonstrate the presence of *B. dysenteriæ* in one-eighth to one-third of the diarrhœal cases submitted to bacteriological examination '.

Asylum dysentery presents an ætiological problem similar to that of summer diarrhœa. There is no doubt that it is frequently a true dysentery, and that the Flexner group is the commonest cause (Vedder and Duval, 1902 ; Eyre, 1904 ; Kruse, Rittershaus, &c., 1907). Shiga's and Sonne's bacilli are also found sometimes, and there is no reason to doubt the occurrence of the Schmitz type. The disease tends to burst out at intervals, and to last for a few years in an epidemic form, after which it subsides and only sporadic cases occur every year, until a new epidemic breaks out. Carriers are the main source of infection (Gettings, 1914–15), and the impaired vigour and insanitary habits of the inmates account for the tendency to spread. The mortality from dysentery in asylums is about 100 times greater than that of the whole population.

The blood-serum of mentally affected persons agglutinates bacilli of the dysentery and enteric groups far more frequently than does the blood of normal persons. It has been suggested that concealed infections of this kind play a part in the ætiology of insanity (Pickworth, 1927).

Clinically abnormal dysenteric infections. The dysentery bacilli are, on occasions, responsible for the production of a syndrome quite unrecognizable as dysentery. Explosive outbursts of intoxication akin to the gastro-enteritis of food-poisoning have been described by Lorenz (1920), where the infecting agent was a Flexner group bacillus of the old Y type ; and sporadic cases of a similar nature have been recorded by Savage and Bruce-White (1925) as caused by Flexner W and Sonne's bacillus. It also appears that, very rarely, a fever of the typhoid type may result from blood-invasion by Flexner bacilli (Warren, 1927). Breakey and Clayton (1926) record the finding of numerous colonies of Flexner (W) in the normal fæcal matter of a child that died with symptoms of a fulminating cerebral infection, for which no other cause was detected. It could not, however, be proved that the bacilli were responsible for the disturbance.

Differences of Pathogenicity and Toxicity to Animals.

The distinction between Shiga's bacillus and the Flexner group is easy to establish by subcutaneous or intravenous injection into rabbits. Whereas half a loopful of a 24-hours Shiga culture given subcutaneously

intoxicates and kills the animal in 4 or 5 days with regularity, a four-times larger dose of Flexner bacilli is borne without disturbance of health (Doerr, 1905). Intraperitoneal injections of adequate doses of both Shiga and Flexner bacilli cause peritonitis and death by septicæmia in these animals, but the dose of Flexner required is usually much larger than that of Shiga ; and some strains are completely atoxic. Exceptions to all these statements are, of course, met with from time to time, since toxicity is a fluctuating character in all pathogenic organisms. Bloody diarrhœa, and even some ulceration, may be produced by Shiga's bacillus with any of these methods of injection ; and also rarely by large doses of Flexner. Fever, wasting, paralysis or paresis of extremities, diarrhœa, hypothermia and death make up the usual syndrome. Post-mortem examination shows acute congestion of the large and small intestines and of the lungs and kidneys. The intestinal mucous membrane and the Peyer's patches are swollen and inflamed. The injected bacillus can be isolated from the watery intestinal contents and from the blood and various internal organs.

Living cultures are not necessary to produce these results ; killed bacilli act in the same manner : a proof that we are dealing not with an infection but with an intoxication. When the injection is not massive enough to cause violent intoxication, the pathological process may manifest itself in a protracted illness accompanied by thickening of the intestinal wall with desquamative and even ulcerative changes of the mucous membrane of the cæcum and large intestine. The picture then approximates to human dysentery ; but the bacilli cannot be found in the intestine, blood or organs ; and this syndrome, too, is apparently due to toxins. Kruse's opinion in 1907 was that nobody had succeeded in giving animals a true dysenteric infection of the human type.

Shiga's bacillus differs strikingly from the Flexner group in that it produces a soluble toxin (culture-filtrate), which kills animals often in very small doses.

Infection of rabbits by oral administration of live dysentery cultures is uniformly unsuccessful unless the animals have been specially prepared by starvation, administration of opium, &c., in which case infection with Shiga bacilli has sometimes occurred (Kazaranow, quoted by Lentz, 1913). There are no records of similar results with other members of the group.

The Sonne type of dysentery bacillus is considerably more toxic to rabbits than the Flexner group, though it hardly equals Shiga's bacillus in this respect (Thjøtta, 1917). Its toxicity, however, is very variable. The evidence as to the effect on rabbits of Schmitz's bacillus is conflicting, but there is no doubt that some strains are strongly toxic (Murray, 1918). *Bacillus alkalescens* is not toxic to rabbits, but the group of miscellaneous dysentery-like bacilli of Table I contains many species which have a strongly toxic action on these animals (Douglas and Colebrook, 1917 ; Winter, 1912 ; Morgan and Ledingham, 1909). The toxicity of the dysentery bacilli to other animals will be considered under the headings of the various species.

Comparative Geographic Distribution.

The distribution of all the types is world wide, but apparently uneven. In Europe, Shiga seems commoner in the south and east, and the Flexner and Sonne types in the North and West. For instance, no case of Shiga dysentery had been recorded in Norway until 1921, whereas the Flexner and Sonne bacilli were very prevalent (Thjøtta and Sundt, 1921[2]). In Denmark the Sonne type was predominant at the time of its first adequate description (Sonne, 1915), and both in Sweden and Scotland this type has been lately found with increasing frequency. Fraser, Kinloch and Smith (1926) isolated dysentery bacilli in the following order of frequency, Sonne, Flexner, Shiga. Nevertheless, practically pure Shiga epidemics may occur in the West, as for instance, in Dublin (Stokes and Bigger, 1920). In some parts of Eastern Asia the Flexner group has been found more abundant than Shiga, e.g. in Malay (Fletcher and Kanagarayer, 1927) and Manchuria (Hoshi, 1925) : also El Tor (Ruffer and Willmore, 1909[1 & 2]) and in the Poona district of India (Manifold, 1926). Amongst the British forces in Mesopotamia in 1918, Shiga and Flexner infections were both common, the latter being rather the more frequent (Ledingham, 1920).

The epidemics in the French armies up to 1917 were due to the Flexner group, though Shiga's bacillus was occasionally found (Vincent and Muratet, 1917). In Central Europe there appears to be no clear preponderance of any type. An epidemic in Metz in 1918 yielded 46 per cent. of Flexner bacilli, 30 per cent. of Shiga and 24 per cent. of Schmitz (Hirschbruch and Thiem, 1918). Vienna in 1920 and Roumania in 1924 harboured Shiga and Flexner bacilli in about equal proportions, and Schmitz infections also occurred in Vienna (Kirschner and Segall, 1920 ; Calalb, 1925[1]). Shiga was the commonest type in Leningrad in 1923, but was accompanied by a fair number of Flexner strains and a sprinkling of Schmitz bacilli. Tribondeau and Fichet (1916) found Shiga much more often than Flexner in the French troops at the Dardanelles, and in Morocco Shiga infections account for 75 per cent. of the bacillary dysentery cases (Job, 1921). In Haiti, West Indies, Shiga was the preponderating, and perhaps the only type isolated by Dickens (1925). American writers report a varying mixture of the Shiga and Flexner types; but they do not as a rule recognize Sonne and Schmitz as true dysentery bacilli ; whence their reports, like those from European countries until very recently, must be considered incomplete. These data can only be taken as giving the most general indication of the distribution of the group. Different epidemics in the same area may be caused by different types. Moreover, the inadequate serological classification frequently adopted makes it certain that a large number of dysentery bacilli have been missed.

Cross-immunity.

In immunological experiments and in human disease do the different varieties of dysentery bacilli give protection against one another ?

The data are scanty and imperfect. The early experiments of Gay (1902–3) led him to envisage Shiga and Flexner bacilli as very closely related in this respect (Flexner and others, 1904). Antiserum made with the one protected also against the other, though it always showed a stronger action against its homologous bacillus. Coyne and Auché (1908) found that whereas Shiga serum had a certain protective power for guinea-pigs injected with living Flexner bacilli, the action of a polyvalent Shiga-Flexner serum was much more powerful. The necessity for polyvalent therapeutic serum was stressed by Shiga in 1908, owing to his discovery that horses, after thorough immunization with, for instance, a mixture of Shiga's bacillus and one Flexner type, could be fatally intoxicated by injections of another type of Flexner bacilli. Amako (1908), on the other hand, seems to have protected mice with Shiga serum not only against Shiga's bacillus but against several races of the Flexner group. The antisera of the latter also showed considerable cross-protection against other Flexner races, though they were always strongest against the homologous organism.

Pribram (1918) published experiments which indicated that Shiga antitoxin had no neutralizing action on his toxic filtrates of the Flexner races D, E and H of Kruse. It should be noted that race E is our Sonne's bacillus. On the other hand, large doses of the antitoxin for Flexner D not only neutralized its own toxin and, almost as strongly, those of other Flexner-group strains, but were even active against lethal doses of Shiga toxin. Some doubt, however, was subsequently thrown on the validity of this work by Prigge (1926), and more evidence is required before the matter can be decided (see also Serum Therapy under the several bacilli).

On the side of active immunity our knowledge is very incomplete. In human beings, an attack of either Shiga or Flexner dysentery does not protect against the other type (Dudgeon and others, 1919 ; Rajchmann and Western, 1917).

We may conclude that whereas some protection against a member of the dysentery group is usually conferred by immunization with other bacilli of the group, the maximum immunity can only be obtained with the actual bacillus against which protection is desired.

Mixed Infections and their Significance.

Though uncommon compared with infection by a single type, mixed infections with two members of the dysentery group have been frequently recorded. Either a Shiga and a Flexner bacillus, or two Flexner-group bacilli, have usually been found.

Gay and Duval (1903–4) made a special investigation on this question by examining large numbers of colonies from ulcers or intestinal contents of three fatal cases of dysentery. In each case they succeeded in cultivating both Shiga and Flexner-group strains ; and in these proportions :

Shiga		4		8		3
———	(1)	—	(2)	—	(3)	—
Flexner		15		22		2

The authors judged from these results that the Shiga and Flexner types were simply alternative forms or modifications of one species. But since later experience has shown the comparative rarity of mixed infections, the chance working of random selection is a more likely explanation. As a rule, individual outbreaks of bacillary dysentery show exclusively, or at least, very great preponderance in one species or type of bacillus (Kruse and others, 1907); but there are many exceptions to this rule (Lunz, 1910).

Mixed infections with bacilli of the dysentery and paratyphoid groups were not uncommon in the Great War, when whole armies, as at Gallipoli, became intensively infected with a wide range of pathogenic intestinal bacteria.

Bacillus alkalescens.

This is the name given by Andrewes (1918) to a bacillus, strongly resembling the Flexner group, which is often found in disordered intestines. It is distinguishable from the Flexner group by (1) its production of strong alkalinity in litmus milk in 7 to 10 days; the colour later becoming slaty, as with *B. fæcalis alcaligenes;* (2) early and intense alkali production in sugar-free media; (3) feeble or no agglutination by Flexner-group sera. Andrewes found that some strains showed a partial and irregular flocculation with some Flexner sera after 24 hours' incubation of the test. The bacilli are feebly antigenic and bad agglutinators, and they give no reaction with the serum of patients who have harboured them for months in their intestines. They have no pathogenic action on rabbits. Michaelis's acid agglutination test is positive; a point which Andrewes and Inman (1919) regarded as distinguishing them from the Flexner group.

This bacillus, or another very closely related to it, had been described previously by Duval and Shorer (1904). Its characters were:—*Milk*, Acid at first, then strongly alkaline; *Glucose*, Acid; *Motility* + or —; *Agglutination* 1 in 500 to 1 in 1,000 with horse's antidysentery serum (type and titre not stated). The bacillus was frequently encountered in the stools of dysentery suspects; and it is interesting to read that it was isolated from several cases whose stools also contained both Shiga and Flexner bacilli.

Transitional, Borderline and 'Paradysentery bacilli'.

Are the divisions of the dysentery group clear-cut and precise, or do they merely represent a convenient grouping of a great range of species, each differing but slightly from the other? And is the group at all closely connected with other known Typhoid-coli subgroups?

Scattered in the literature there are to be found a number of descriptions of bacilli of intermediate nature, i.e. belonging serologically to one division of the dysentery group, and culturally to another. For instance, Konrich in 1908 isolated a bacillus which gave all the biochemical reactions of the Flexner group, but was agglutinatively a Shiga. This combination of properties was confirmed by Sonne (1915), which shows that the organism

had not changed in seven years. It appears to prove that Shiga bacilli can very rarely acquire the power of acidifying mannitol to an extent comparable with that of the Flexner and Sonne groups. This is much less difficult to understand if we accept Winter's (1912) view that Shiga has normally a slight (masked) action on mannitol. It is to be noted, however, that Konrich's bacillus was not proved to be the cause of a dysenteric infection.

We have recorded above the temporary absence of mannitol fermentation in strains of the Flexner group (p. 165), which gives them the false appearance of being intermediate types. Again, it has been alleged by Amako (1908) and Calalb (1925[1, 2 & 3]) that bacilli serologically of the Shiga type may produce indole; but more confirmation is required before we can accept these observations as valid.

Late-lactose-fermenting strains serologically identical with Sonne's bacillus, but possessing also a strong agglutinative and absorptive relationship to the Y type of the Flexner group, have been described by Murray (1918). He also found Flexner X (Toner) serum to agglutinate lactose-fermenting strains to a varying degree. Further, we may recall in this connection a tendency often noticed in the old 'Y' (Hiss and Russell) to alter the colour of lactose media in the acid direction. These observations speak for a relationship between these two groups closer than either of them possesses to Shiga's or Schmitz's bacilli. The last-named organism has been regarded by some as intermediate between Shiga and Flexner, for it resembles the former in having no action on mannitol, and the latter in producing indole. But since it is serologically quite distinct from both, it seems more reasonable to regard it as an independent type.

Alleged transmutation of species. If the various types of dysentery bacilli were mere temporary modifications of a single stock, as some have claimed (see above, under Mixed Infections), one would expect that transmutation of type, either spontaneous or induced, would be a not uncommon occurrence. But if we pass over the relatively common variations of fermenting power, already described, and restrict our attention to fundamental serological characters, we find overwhelming evidence of their stability. A Shiga bacillus does not change into a Flexner, Schmitz or Sonne type; nor vice versa. Such evidence as exists in favour of the theory of transmutation is unconvincing.

Thjotta (1920) has put on record an interesting change that occurred in the biochemical properties of an organism that he isolated. The first series of tests showed it to be a typical Morgan's bacillus; but on repeating the tests a year later, the characters were those of a Flexner. The bacillus, however, showed no serological connection with any member of that group.

Some remarkable and widespread mutations have been described by certain workers in cultures of dysentery bacilli; but in the absence of adequate confirmatory observations on pure-lines of bacilli they cannot be considered as proved. In this category we may place the observations of Schmitz (1919), who claims to have followed the development in a

culture of his own bacillus of a whole series of widely differing species, including *B. coli*, paratyphoid bacilli and mannitol-fermenting dysentery strains. The discovery of gas-producing and other aberrant Shiga-variants by Isabolinsky and Gitowitsch (1926), and the transmutations of Schmitz's bacilli seen by Calalb (1925), are probably of the same type.

Based upon the conception of transmutations there is a theory held by a few writers (Seligmann, 1917) that pathogenic dysentery bacilli arise afresh in each epidemic period from non-pathogenic coli-group organisms. The theory is supported by (1) the frequent failure to detect any carriers of dysentery bacilli in a population in the pre-epidemic period, and (2) the isolation of a number of aberrant coli-dysentery group bacilli in the early stages of epidemics. It need hardly be said that a theory so directly in conflict with the current and well-grounded belief in the relative stability of fundamental bacterial characters needs much more concrete evidence before it can claim general acceptance.

'Dysenteroid' and 'Paradysentery' Bacilli.

No one who studies the literature of this subject can fail to be impressed by the multitude of bacterial species that have been described as causing, or at least, as being associated with, diarrhœal disturbances of a more or less dysenteric type. The great majority of these, and the only ones we need consider here, are members of the typhoid-coli group which lie somewhere in the scale of characters between *B. coli* and *B. dysenteriæ*, sometimes approximating to the one, sometimes to the other. Indeed, from the purely bacteriological point of view, it is impossible to draw any sharp line between the organisms at the dysentery end of the scale and the dysentery group itself. And when we take into account the varying phases of bacilli, their adaptability and their frequent changes of fermenting power and serological properties, we are forced to admit that we do not possess the knowledge necessary for a permanent and satisfactory classification.

In Table II there are shown a selected few of the 'coliform' or 'paradysentery' bacilli that have been isolated from the human intestine, mostly in diarrhœal or dysenteric conditions. Similar bacilli may sometimes be found in healthy excreta of men and animals, and in flies (Morgan and Ledingham, 1909; Graham-Smith, 1911–12). The data given in the table are incomplete, and in most cases represent only a perfunctory examination of the organism. The characters of Morgan's bacillus, however, rest on a sounder basis, and will receive further notice at the end of this section.

Attention may be called also to *B. coli anaerogenes*, which has been found by a number of workers in their search for dysentery bacilli. It is impossible to be sure whether it is a species or a group of species. The different strains seem inconstant in their production of gas from lactose, for this has sometimes been described as positive and at other times as negative. Similarly, the acidification of milk may set in rapidly or be

TABLE II.

Some Borderline organisms ; ' paradysentery ' bacilli, &c.
(All morphologically and culturally members of the typhoid-coli group.)

Description of bacillus	Glucose	Lactose	Mannitol	Dulcitol	Saccharose	Milk	Indole	Motility
Morgan and Leding-ham's group 15.	O	O	O	O	O	O or Alk	O	+ or O
Morgan and Leding-ham's group 5.	A	O	O	O	O	A or O — sl Alk	+	+ or O
Douglas and Cole-brook's group 8.	A	O	A	A	O	O	+	O
Douglas and Cole-brook's group 6.	A	O	A	A	A	O	+	O
B. coli anaerogenes (Nabarro - Cruick-shank).	A	A or sl G	A	?	A	AC	+ or O	+ or O
Douglas and Cole-brook's group 9.	A	A	A	A	A	AC	+	O
Morgan's No. 1 ..	A sl G	O	O	O	O	O or A — sl Alk	+	+ or O
Paradysentery bacil-lus* (Loewenthal, 1912).	AG	O–A	A	O	O	A–Alk A–C	+	O
Morgan and Leding-ham's group 14.	AG	O	AG	O	O	A–Alk	—	+ or O
Morgan and Leding-ham's group 12.	AG	O	AG	O	AG	AC	+ or O	+ or O
Morgan and Leding-ham's group 10.	AG	O	AG	AG	AG	A–AC A–Alk O–Alk	+ or O	+ or O
Paradysentery bacilli (Nègre, Sergent and Foley, 1916).	AG	AG	AG	?	?	AC	?	+ or O

* The characters given are those of the freshly isolated bacillus (see text).

A = Acid. G = Gas. C = Clot. Alk = Alkaline. sl = Slight. + = Positive reaction. O = No change. A–Alk = Acid changing to Alkaline.

delayed for 7 to 10 days, and coagulation may or may not occur. The chief interest of the bacillus for our present purpose is that in its non-motile form, if no gas is produced in lactose, it is culturally and biochemically indistinguishable from Sonne's dysentery bacillus. The two species can then only be distinguished by serological tests.

The various bacilli taken from Morgan and Ledingham's (1909) list and from Douglas and Colebrook (1917) lay no claim to pathogenic importance, but simply illustrate the complexity of the intestinal flora. Many of them are strongly toxic to rabbits.

The paradysentery bacilli of Nègre, Sergent and Foley (1916) though clearly of the coli group, were so frequently associated in Algeria with a mild but typical dysentery, and gave rise to such a high titre of agglutination (up to $\frac{1}{1000}$) in the patient's blood, that the authors believed them to be the cause of the disease. The bacilli differed strongly from *B. coli* in the appearance on agar of the colonies, which in size and shape were far more like those of dysentery bacilli than of *B. coli*. The same organisms were found in the stools of persons suffering from typhoid-like symptoms, or from chronic colitis.

The paradysentery bacilli of Loewenthal (1912), which he isolated from cases of acute enteritis, showed two distinct phases. Directly after isolation, their characters, as given in Table II, placed them near to *B. coli anaerogenes*, but after a period of cultivation the colonies were seen to produce daughter-colonies possessing such increased powers of fermentation that they could no longer be distinguished from *B. coli* (cf. *B. coli mutabile*). Further, a more prolonged incubation of the freshly isolated strain in the carbohydrate media gave ultimately the same result.

Deycke and Reschad (1904) also grew bacilli resembling the first phase of Loewenthal's organism from a number of cases in a dysentery epidemic in Constantinople. The bacilli were found either with or without Flexner-group bacilli. The authors did not observe any change in the direction of *B. coli*, but when later a strain fell into Kruse's hand, it proved to have the characters of that organism (Kemp, 1907). Still more frankly of the Coli group were the bacilli of Abe (1908), which were grown frequently in pure culture from dysenteric stools and agglutinated to a titre of several hundreds by the patient's blood.

The present state of our knowledge does not allow us to assess the ætiological importance of these various bacilli in diarrhœal conditions: they may be mere saprophytes, causing agglutinin production by their harmless passage through damaged intestinal mucosa, or they may be the true cause of the condition. One and all are distinguishable from the true dysentery bacilli by their serological constitution, and by at least one biochemical test.

Morgan's (No. 1) bacillus.

This organism was described by Morgan (1906) as the most frequent of the non-lactose-fermenting bacilli of the typhoid-coli group found in the summer diarrhœa of infants.

Cultural and Biochemical Characters.

The main features of the bacillus are shown in Table II (p. 178). It is a non-lactose-fermenting Gram-negative bacillus of the size of a dysentery or colon bacillus. Ordinarily it is motile, but it can occasionally show immobility. It produces acid and gas in glucose (lævulose and galactose), but the gas may be formed in such small quantity as to be detectable only in deep agar culture or even to be missed altogether. The bacillus produces indole (though a negative reaction has occasionally been reported), and does not coagulate milk nor liquefy gelatin. It blackens lead acetate, and produces a strong fæcal odour in cultures (Tribondeau and Fichet, 1916). A list of fermentation-reactions with 25 different substances is given by Dungal (1927).

Variation. The reactions in sugars other than lactose and glucose are not sufficiently regular to be of use in classification, though several attempts have been made to subdivide the group on this basis. Tribondeau and Fichet (1916) found that Morgan strains, when freshly isolated, could be arranged in three biochemical groups, according to their action on mannitol, maltose and saccharose. But when they were all tested again some time later, none of them fermented anything except glucose. As regards motility, reports vary. The majority agree in calling the bacillus motile, but none of Douglas and Colebrook's (1917) 8 strains showed any movement. Tribondeau and Fichet saw 2 non-motile strains out of a total 13. They demonstrated peritrichous flagella, 1 to 5 in number, in all races. When we consider that a non-motile temporary modification of *B. typhosus* and similar motile organisms is not uncommon, the variability of Morgan's bacillus need not surprise us. Thjotta (1920), on retesting a number of typical Morgan strains 18 months after isolation, found that the majority had lost their motility. Of these, two no longer produced any gas in glucose, but had acquired the power of acidifying mannitol. They thus showed all the biochemical characters of Flexner's bacillus, and could not have been distinguished from 'inagglutinable' strains of that group. According to Lewis (1911–12), after Morgan No. 1 the commonest non-lactose-fermenter found in summer diarrhœa is a bacillus differing from the last named only in its failure to produce gas. Thjotta's work shows that these may merely be modifications of Morgan's bacillus.

Serological Reactions.

The races of this bacillus are serologically diverse; that is to say, the serum made with one typical strain does not agglutinate other typical strains. In this respect Morgan No. 1 resembles *B. coli* and the influenza bacillus (Morgan and Ledingham, 1909; Thjotta, 1920; Kligler, 1919; Dudgeon and others, 1919).

The blood-serum of patients from whose stools the bacillus has been isolated does not, as a rule, give a positive agglutination-reaction with the homologous bacillus or with stock bacilli (TenBroek and Norbury, 1915; Tribondeau and Fichet, 1916; Douglas and Colebrook, 1917; Davison,

1920). It is true that Morgan and Ledingham (1909) detected a supernormal agglutination of the bacillus in fully half of their cases; in fact, in 80 per cent., if only late cases and convalescents are considered. The reactions occurred in low dilutions, 1 in 20 to 1 in 40, whereas normal human sera rarely reacted at 1 in 10. But in the light of the failure of other workers to confirm these observations, and considering that the bacillus is readily agglutinogenic on inoculation into animals, we must admit that the serum-test provides no convincing evidence of its infectivity.

Pathogenic Action on Animals.

Morgan (1906) succeeded in infecting young rats, young rabbits and monkeys by the mouth, but had no success with intravenous or intraperitoneal injections. Injections of living cultures, autolysed cultures and old culture-filtrates are toxic to young rabbits, rats, guinea-pigs and goats. Intravenous injection may cause congestion, exudation and occasionally ulceration in the small intestine (Douglas and Colebrook, 1917). Peritonitis and inflammation of the intestinal mucosa follow intraperitoneal injections into guinea-pigs. After intravenous administration of one twenty-fifth of a loopful of a young agar culture, guinea-pigs may die in about 10 days with paralysis (Tribondeau and Fichet, 1916).

Virulence is soon lost in culture, but can be restored by passage through animals. Soluble exotoxin is not produced.

A spontaneous epidemic of Morgan infection in tame laboratory mice has been recorded by Wilson (1927). An attempt to start a similar epidemic by feeding was unsuccessful; which suggested that a second factor, such as diet, enters into the ætiology of infection with this bacillus.

Pathogenic Action on Man.

There is no conclusive evidence that Morgan's bacillus is the cause of intestinal disturbance or any other disease in man. The widespread belief that diarrhœa and even dysentery may be caused by its multiplication in the intestine rests on the impressions of bacteriologists, who are naturally prone to attribute ætiological significance to any bacillus that appears in large numbers in a disordered colon. These impressions may or may not be true, but the failure of the agglutination test is against their truth, and there are no records of accidental or intentional infections with pure cultures to prove the point directly. In favour of the infectivity of his bacillus Morgan quotes the suggestive case of a nurse who contracted diarrhœa while attending children suffering from the same disorder. In her stools the bacillus was found in pure culture.

Distribution of the Bacillus.

Morgan's bacillus is a frequent inhabitant of the intestines of various animals and insects, such as cows, mice, cockroaches and flies. In healthy human beings it is not common except in epidemic periods, when it may be broadcast through a population. Lewis (1911 12) found it in 17 out

of 28 samples of Birmingham milk. Of the many varieties of non-lactose-fermenters found in flies, Morgan No. 1 is the only one that occurs frequently in flies from houses infected with diarrhœa, and rarely in flies from uninfected houses (Graham-Smith, 1911-12). A greater proportion of flies are infected with non-lactose-fermenters in July and early August than at any other time of the year. Morgan gives the following figures for the frequency of his bacillus in the summer diarrhœa (S.D.) of infants in 1905 and 1906 :

1905—58 cases of S.D. .. .. Morgan No. 1, 28 times.
1906—34 cases of S.D. .. .. Morgan No. 1, 15 times.

Lewis (1911-12) found it in 95 per cent. of cases of infantile diarrhœa, and in 38 per cent. of stools of healthy children of the same population. In some years, however, the bacillus has been conspicuous by its absence. For instance, in 1921 Nabarro (1923[2]) examined 107 cases of summer diarrhœa without coming across Morgan's bacillus at all. He concludes that it is certainly not the specific cause of the disease.

The distribution of the bacilli in the bodies of children after death from infantile diarrhœa was found by Morgan to be as follows :

No. of cases, 16. Colon, 13; jejunum, 1; spleen and mesenteric glands, 3; liver and bile, 2; lung, 1; kidneys and urine, 3; heart's blood, 3.

During the Great War, Morgan's bacillus was found widely distributed in soldiers suffering from intestinal disorders; and it is not infrequently encountered at the present time in asylum dysentery and other severe diarrhœas of adults.

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B. dysenteriæ (Shiga).

(Synonym. *Eberthella dysenteriæ*).

By L. S. Dudgeon.

Definition and Description.

B. dysenteriæ (Shiga) is a cause of epidemic, endemic and sporadic dysentery in all parts of the world. Its characters may be briefly summarized as follows :

Rod-shaped bacillus—may be a cocco-bacillus. Average size in ordinary conditions ; 0·4 to 0·6 by 1·0 to 3·0μ. Non-motile. No flagella. No spores nor capsule. Gram-negative. Stains readily with ordinary dyes. Acidifies monosaccharides without gas formation. Gelatin ; grows well at 22° C., but does not liquefy. Milk ; acidification and subsequently alkalinity ; no clotting occurs. Agar ; grows readily as small greyish semi-transparent colonies. Broth ; uniform turbidity. Optimum temperature ; 37° C. Indole ; negative.

Differentiation from Allied Bacteria.

B. dysenteriæ (Shiga) is readily differentiated culturally from Flexner's bacillus because it never ferments mannitol or saccharose, it is absolutely distinct serologically, and it is more highly toxic for rabbits and horses.

The bacillus isolated from cases of dysentery in Macedonia which Dudgeon and Urquhart (1919) described as para-Shiga— is culturally identical with *B. dysenteriæ* (Shiga), but serologically it can be readily differentiated from this bacillus. It differs from Schmitz's (para-Shiga+) bacillus, which forms indole and is less toxic for animals. The Shiga bacillus and Schmitz's organism are distinct serologically.

Proof of the Connection of the Bacillus with Dysentery.

Shiga (1898) recognized at the outset that the bacillus of bacillary dysentery must fulfil four essential ætiological conditions—(1) it must occur constantly, (2) it must be a species not normally present in the organ concerned, (3) it must be pathogenic and produce in experimental animals lesions similar to those from which it was obtained, (4) it should be agglutinated with the blood of patients recovering from the disease. All these requirements were fulfilled by the bacillus of Shiga. Since his work was published his bacillus has been isolated from cases of dysentery occurring in all parts of the world.

Morphology.

The Shiga bacillus is a rod-shaped organism with rounded ends which varies in size from 0·4 to 0·6 by 1·0 to 3·0μ. It is often present as a 'cocco-bacillus' in film preparations of dysenteric stools and in beef broth from recently isolated cultures. In my experience filaments and involution forms occur but are very uncommon. The bacillus sometimes shows polar-staining, in which case it may be confused with the cocco-bacillary form.

The organism is always Gram-negative and stains readily with any of the usual dyes. It does not form spores, and capsules are never seen. I have never seen a motile Shiga bacillus: young beef broth, veal broth, peptone water and agar cultures have been inoculated from strains of the bacillus recently isolated from cases of dysentery and from old laboratory cultures, but no motility has been observed. Flexner (1907) stated that the organism is usually not motile in cultures made from cases of human dysentery unless the examination is made from young cultures, 16 to 18 hours old in the first generation. This reservation, however, I cannot confirm.

Cultivation.

The optimum temperature for the cultivation of the Shiga bacillus is 37° C. The growth is more restricted at room temperature, and Flexner (1907) states that it is arrested at 5 to 6° C.

The organism grows well if the reaction of the medium is pH 7·5. It is highly sensitive to reactions which are on the acid side of the figure, but will grow in a medium with a reaction of pH 11. Owing to this fact, Dudgeon and his colleagues (1919) adopted a method by which alkali is added to all samples of dysentery stools, if delay in the bacteriological

examination is probable. The reduced alkalinity, or even acidity, which may occur in hot countries when the bacteriological examination of the fæces is delayed for several hours considerably diminishes the probability of isolating the Shiga bacillus. I have found that the growth of the Shiga bacillus may be definitely greater if the alkalinity is increased. A growth of the Shiga bacillus may be as great as 1,100 million per c.cm. in beef broth with a pH of 11 after 24 hours' incubation at 37° C., but only 700 million per c.cm. if the reaction is 9, other conditions being similar. It will grow anaerobically, but to a less degree than aerobically, and stock cultures give the same cultural reactions by either method. Cultivations from the tissues, exudates, or fæces should be incubated aerobically at 37° C.

The Shiga bacillus grows well on the ordinary media such as agar and beef broth, but is readily overgrown by *B. coli*.

Surface colonies isolated from the stools on any agar medium are small, delicate and semi-transparent ; the outline is regular, the surface smooth and there is little tendency to enlarge as age advances. The colonies are blue on litmus lactose agar, neutral on Endo's basic fuchsine lactose agar or MacConkey's neutral red lactose agar, and a very pale pink on phenol-red lactose agar. It is most important that the reaction of the medium should be of pH 7·4 or 7·5. Although the Shiga bacillus grows well in beef broth at pH 11·0 as previously stated, yet no growth may occur on litmus lactose agar with the same reaction. On gelatin plates the colonies are small, bluish-white and semi-transparent. The margins may be regular or wavy and irregular.

The primary isolation of the bacillus is most readily carried out on litmus lactose agar at pH 7·4 or 7·5, on which it grows readily. It can be kept alive on agar at room temperature in the dark for prolonged periods, provided that the medium has the correct reaction.

The organism does not readily show evidence of autolysis.

Arkwright (1921) described the characters of rough and smooth colonies which are seen on the surface of agar plates. He examined nine strains of the Shiga bacillus and obtained smooth ' S ' and rough ' R ' forms in eight, and in one ' R ' forms only. He found that the ' S ' form emulsified readily in normal saline and made a suitable emulsion, while the ' R ' forms agglutinated in normal saline. The ' S ' forms have a uniform turbidity in broth with a very slight deposit ; with the ' R ' forms the broth is clear as the bacilli are deposited at the bottom of the tube. The ' S ' forms are smooth, round, domed, shiny and translucent colonies on the surface of agar, while the ' R ' colonies have a rough, irregular outline, a rough or dull surface, and are slightly opaque. Frequent subcultures in broth appear to favour the formation of ' S ' colonies, and prolonged periods without subculture the ' R '. The ' S ' forms agglutinate in large clumps with specific antisera, while the ' R ' forms only agglutinate in fine clumps. The ' R ' forms are difficult to pick off the media because of their coarse cohesive characters.

Biochemical Reactions.

The Shiga bacillus does not digest protein substances and never forms indole. Gelatin, inspissated blood-serum and Dorset's egg medium are not digested.

It does not lyse red blood corpuscles : every strain which I investigated during the War was non-hæmolytic.

The Action on Carbohydrates, Milk and Potato.

Cultures of the Shiga bacillus isolated from cases of dysentery in various parts of the world and stock laboratory cultures all give the same carbohydrate reactions. This fact is one of the most instructive features of this important organism : so much so that irregular results suggest impurities in the media or other errors of technique. The bacillus ferments dextrose and the monosaccharides without gas formation. Mannitol, saccharose, lactose, maltose and dulcitol are unaffected. Many of the earlier records on the bacteriology of bacillary dysentery can be disregarded because it was not realized that the Shiga bacillus never ferments mannitol. It is this character which is its chief cultural distinction from the Flexner group. In mannitol, maltose and saccharose media with phenol red as the indicator, it may show a definite alkaline reaction in 7 to 12 days at 37° C. Rough and smooth colonies give the same carbohydrate reactions.

Milk is acidified, though not strongly, in 24 to 48 hours ; it is not clotted or thickened. An alkaline or less frequently a neutral reaction succeeds the acidity.

Potato. A fine semi-transparent growth forms on the surface of potato, which may acquire later a dirty grey or brownish colour.

Serological Reactions.

Production of Antibodies in Animals and Man.

The immunization of man and animals with the Shiga bacillus has to be carried out with considerable care owing to the toxicity of cultures, living or dead. Animals rapidly lose weight and unless the dosage is carefully graduated succumb to the live or dead bacillus, more especially if it is injected intravenously. In man subcutaneous inoculation with dead cultures of the Shiga bacillus produces an extensive local reaction with pyrexia and general bodily disturbance, so that an individual may be incapacitated for several days. Formolized vaccines are toxic for animals, but live cultures and heat-killed vaccines are infinitely more so. Guinea-pigs are less responsive to active immunization than rabbits.

The Shiga bacillus produces a diffusible exotoxin which can be separated from fluid cultures by filtration.

If full-grown rabbits are injected intravenously with small doses, such as 25 million bacilli in a formolized saline emulsion of a 24-hours' growth on agar, immunization can be efficiently carried out, provided the doses are spaced correctly and given only to animals showing an increase in weight. Rabbits gradually immunized with live or attenuated cultures,

heat-killed or formol-killed vaccines, develop immune substances, but the maximum agglutinin titre of the serum in my experience is much less than that obtained with many other specific organisms. Brunckmann (1926) considers that increased agglutinability of the Shiga bacillus is obtained by growing it in dextrose and to a somewhat less extent in galactose. Even emulsification of the bacillus in a solution of either sugar, but especially dextrose, is as valuable as growing the bacillus in the sugar.

Agglutinins—' Inagglutinable ' Strains.

Benians (1919–20) made the following experiments on the inagglutinability of *B. dysenteriæ* (Shiga). A 24-hours' growth on an agar slope was emulsified in 10 c.cm. of sterile 5 per cent. mucilage of tragacanth and 2 c.cm. of this emulsion was injected subcutaneously into the flank of a guinea-pig. One month later the inflammatory mass that formed was tapped and muco-purulent fluid was evacuated, from which a pure culture of the Shiga bacillus was obtained. The bacillus was true in its fermentative and serological activity. At the end of another month the mass was again tapped and from the pus two kinds of colonies were obtained. (1) Moderately large, clear and rounded colonies which proved to be a typical Shiga bacillus. (2) Smaller colonies which were raised, opaque and granular. The bacillus from these colonies gave the cultural characteristics of the Shiga bacillus, but was inagglutinable. In broth it formed a deposit with a clear supernatant fluid.

A broth culture of this inagglutinable strain which had been kept at 37° C. for 3 weeks was plated out and three types of colonies were obtained. (A) A typical Shiga bacillus culturally and serologically, but few in number. (B) Colonies ' like ' *B. dysenteriæ* (Shiga), but much smaller; with the same cultural reactions, but inagglutinable. Abundant growth was obtained. (C) Inagglutinable opaque colonies, few in number, with cultural characters the same as the Shiga bacillus. Two rabbits were immunized with strains A and B. Immunization with strain A afforded protection against A and B, but no agglutinins were formed for B. Immunization with strain B produced protective immunity to A and B, but no agglutinins were formed. The inagglutinable strain did not absorb the agglutinins from a standard Shiga antiserum, but was absorbed by the agglutinable strain. Both strains were highly pathogenic for rabbits.

The bacillus which Dudgeon and Urquhart called para-Shiga— (1919) was isolated in Macedonia during the war from 11 cases of dysentery. This organism corresponded to the true Shiga bacillus culturally, but was inagglutinable with a Shiga antiserum. The colonies on agar media were like those of the Shiga bacillus and the Shiga bacillus was not obtained from cases from which this organism was grown. B. para-Shiga— was not agglutinated by and would not absorb Shiga antisera. Immune para-Shiga— rabbit serum did not agglutinate the Shiga bacillus and the Shiga bacillus would not absorb the agglutinins from a para-Shiga—

immune serum. It is possible that the bacillus is an inagglutinable strain of the Shiga bacillus, but there is nothing to prove that it is, and serologically it is entirely dissimilar. Cultivation on artificial media always failed to reveal any true Shiga colonies from plating of the para— bacillus. Seligmann (1917) from his experiments on 'cross agglutination' found that Shiga bacilli whether inagglutinable, feebly agglutinable, or 'atypical', produced antisera which agglutinated all Shiga bacilli. Taylor (1919) isolated from a fly captured in a hospital in Macedonia an inagglutinable strain which was culturally identical with the Shiga bacillus. Taylor unfortunately had none of the para-Shiga— serum when he isolated this organism, and, therefore, it was impossible to prove whether it was definitely B. para-Shiga— .

Co-agglutinins in human sera for the Shiga and Flexner bacillus are referred to later under Practical Diagnosis. In the antisera of animals immunized with the Shiga bacillus only true specific agglutinins are formed.

Methods. The two chief methods of studying the presence of Shiga agglutinins in immune sera are as follows : (1) Emulsions of live cultures are mixed with varying dilutions of the antiserum and the results observed microscopically. (2) Sensitive veal-broth antigens, or broth or agar antigens killed with formalin are mixed with dilutions of the antiserum, and the tubes, each containing 1 c.cm., are incubated in a water bath at 52° C. for 4 hours. The advantage of the latter method is that with formolized antigens non-specific agglutinins are much less in evidence.

I have found that subculture of the Shiga bacillus on agar daily, or even more frequently so that 10 subcultures are made in the week, and subsequent emulsification of the organism in normal saline containing 0·1 per cent. formalin, makes as efficient an antigen as veal broth. These antigens may remain constant for more than 12 months. The most satisfactory opacity is 500 million bacilli per c.cm. as judged by Brown's opacity tubes. Shiga agglutination is finely granular at the commencement of the reaction. In 4 hours at 52° C., if the reading is taken after the tubes have been at room temperature for 15 minutes, a perfectly clear end point is given. This is taken as the last tube in which agglutination can be detected with a hand lens against a black background. I have found that Shiga antigens prepared in this way give remarkably constant results with the same sample of Shiga antiserum. Normal rabbit sera do not react with Shiga antigens in a dilution of 1 in 20, and with human sera a reaction of 1 in 40 at the end of 4 hours at 52° C. with a Shiga antigen must be regarded as pathological.

Precipitin and complement-fixation reactions have been studied with Shiga antigens and immune sera, but the methods are more laborious and the results obtained are not more satisfactory than with agglutinins.

Pathogenic Action.

Animals are extremely sensitive to the Shiga bacillus, whether injected intravenously, intramuscularly or subcutaneously. Rabbits are readily

killed with live cultures, vaccines or toxins. Shiga toxin when injected into rabbits by any of the usual methods may produce a hæmorrhagic enteritis with a pseudo-membranous exudate on the surface of the mucosa. If rodents are fed on a culture of the Shiga bacillus the symptoms or lesions of bacillary dysentery seldom develop, but if a culture is injected into rabbits intravenously, intraperitoneally, or subcutaneously diarrhœa may develop and the disease end fatally. In such cases Peyer's patches may be enlarged and hæmorrhagic, the walls of the cæcum swollen, œdematous and hæmorrhagic, and the mucosa covered with a false membrane. Animals which survive for a few days may develop ulceration of the bowel. The bacilli may be isolated from the intestinal lesions when an inflammation of the intestinal wall has followed intravenous injection of the bacilli, and also from the blood-stream. By no means infrequently paralysis follows the injection of live bacilli, toxins or vaccines into rabbits. It usually occurs quite suddenly in the hind quarters 2 or 3 days or longer after the inoculation and rapidly becomes more extensive before the death of the animal. Hæmorrhages into the spinal cord and nerve-cell degeneration have been found in such instances.

Dudgeon and Urquhart (1919) experimented with recently isolated cultures of the Shiga bacillus, which they injected into the lumen of the intestine in rabbits. In these experiments the dosage varied from 1,500 to 7,500 million bacilli obtained from young agar cultures suspended in saline. In one instance 1·5 c.cm. of blood and mucus from a case of acute Shiga dysentery was injected into the lumen of the gut with an equal quantity of saline. Clinical evidence of acute dysentery was obtained in the case of one rabbit only, which died in 24 hours. The bowel was not ulcerated, but showed evidence of acute inflammation along its course. No agglutinins were formed in the serum of these rabbits. It is a matter of considerable interest that virulent cultures of the Shiga bacillus or the blood and mucus obtained from a case of acute Shiga dysentery when introduced directly into the lumen of rabbits' intestines, failed to excite acute bacillary dysentery, with the one exception already referred to. Similar cultures, when injected intravenously, proved fatal within three days, in spite of previous active immunization with Shiga vaccines which had excited a high agglutinin content in the sera. In one instance a positive blood-culture was obtained from the living animal 24 hours after inoculation.

The Shiga bacillus produces an endotoxin and an exotoxin which can be separated from fluid cultures by filtration methods. Conradi (1903) added normal saline to a 24-hours' surface growth on agar and allowed it to remain in contact with the bacilli at 37° C. for 24 to 48 hours. The saline suspension was then filtered through porcelain candles and concentrated. A toxin was obtained which in doses of 0·1 c.cm. was fatal to rabbits in 24 hours. Rosenthal (1903) grew the bacillus in weak alkaline broth for about three weeks at 37° C. 0·2 and 0·1 c.cm. of this broth,

after filtering, killed rabbits when injected subcutaneously in 24 to 48 hours. The clinical symptoms were paresis of the hind legs and later of the forelegs, frequent diarrhœa, rapid fall of temperature, considerable loss of weight and collapse. Rosenthal obtained a protective antiserum from the rabbits injected with this toxin.

Sudmerson Runge and O'Brien (1924), prepared a Shiga toxin by growing the bacillus on nutrient agar for 48 hours, washing off the growth in distilled water, heating just to the lethal point, i.e. 58° to 60° C. for 10 minutes, centrifugalizing and drying rapidly in a desiccator. The dried scales were finally ground so as to make a homogeneous powder, of which 0·04 mgm. was fatal to mice of 20 gm. When injected intravenously 0·005 mgm. might prove fatal, but the result was uncertain.

Dudgeon and Hope Simpson (unpublished) prepared a Shiga toxin as follows : Four agar slopes were heavily inoculated from an agar culture and incubated for 24 hours at 37° C. Sterile distilled water was then added up to the top of the agar slopes and the tubes were re-incubated for 24 hours at 37° C. The distilled water and bacilli were poured off and filtered through a German Berkfeld W candle. The clear filtrate was poured into a sterile Petri-dish and evaporated to a sticky residue over sulphuric acid in a desiccator, in which a vacuum had been created. The powder obtained was dissolved in 2 c.cm. of sterile saline containing 0·1 per cent. of formalin and centrifugalized to remove any foreign particles. 0·5 c.cm. injected intravenously killed full-grown rabbits in 40 hours.

Lüdke (1906) obtained large surface growths of the Shiga bacillus on agar in 24 to 36 hours at 37° C. The growth was washed off in normal saline, centrifugalized, and the emulsion dried in a vacuum at room temperature. The amount was very small, seldom more than 0·1 gm. The dried powder was frozen and the bacilli were ground up with a hand pestle and mortar. To the pasty mass so obtained, 20 to 40 c.cm. of a sterile solution of normal saline was added, and the whole was filtered. A clear solution was obtained of which 0·5 to 0·2 c.cm. killed full-grown rabbits in 18 to 24 hours, 0·1 c.cm. killed rabbits in 48 hours in some cases, and in others produced symptoms such as occur from the injection of live dysentery bacilli. The best results were obtained from the intravenous injection of 0·05 c.cm. of the extract, which killed rabbits in 36 hours. Guinea-pigs which had received an intraperitoneal injection of 0·5 to 0·1 c.cm. died in 1 to 3 days. The most characteristic symptom was rapid loss of weight.

The potency of these extracts appeared to be limited by time, as after eight days on the ice the fluid was much less toxic. After the injection of sublethal doses a further dose killed the animal, but no antibacterial or antitoxic bodies were formed in the blood-serum. No agglutinins were present in the serum of rabbits after one sublethal dose. A hyperleucocytosis followed a subcutaneous injection.

Dudgeon and Hope Simpson in 1927 made a large number of experiments on rabbits with a Shiga toxin prepared from cultures grown for 1 day, 10 days and 1 month at 37° C. in flasks containing 200 c.cm. of beef broth in which there was 1 per cent. of bacto-peptone (May and Baker). The reaction of this medium was pH 7·5. The flasks were capped with jackonet at the end of 24-hours' growth at 37° C. The growth was filtered through German Berkfeld candles (W), by means of a water suction-pump. The toxin was stored in sterile bottles; 0·1 per cent. of formalin was added, and the bottles were capped with waxed corks. Three grades of toxin were investigated, (1) one month, (2) 10 days, and (3) 1 day. The 1 month is by far the most toxic, and the 1 day toxin is only very slightly so for rabbits, as large doses can be injected without killing the animals. Rapid and progressive loss of weight is a certain indication of the toxic action of Shiga toxin in rabbits, and is definite evidence of ill-health before any other clinical manifestation is observed. It occurs in rabbits injected with the living bacillus, toxins or vaccines, and is a most striking feature of chronic Shiga dysentery in man. In rabbits which recover, the weights 8 to 10 days after inoculation may be unchanged, or a very slight gain may be recorded. Flasks of beef broth grown for one month at 37° C. were plated so as to obtain surface colonies on agar plates. Rough and smooth colonies were readily distinguished, but the rough colonies were in excess. Subcultures of each type were made in tubes of beef broth, and from these flasks of beef broth were inoculated for the preparation of rough and smooth toxin. Rabbits injected with Shiga toxin prepared by this technique soon show evidence of intoxication, but no difference was shown in toxicity between the rough and smooth toxins. The obvious signs of ill health were loss of appetite, or failure to take food, and rapid and progressive loss of weight until a fatal result occurred; paralysis of the hind legs or general paralysis sometimes preceded the fatal termination. In each experiment the weight of the rabbit was taken each day at the same time before the morning feeding, and the afternoon feed of fresh vegetables was given always at the same hour. The blood was collected for the various examinations required by the same technique in each case. Post-mortem examinations were made very shortly after death and portions of the various viscera were taken for microscopical examination.

Blood and Tissue Changes in Shiga Toxæmia in the Rabbit.

The blood was obtained by puncture of a vessel in the rabbit's ear and the total number of leucocytes were counted by the Strong-Seligmann method. Film preparations of the blood were made at the same time and stained with Leishman's stain. Every care was taken to collect the blood from vessels with healthy walls and normal blood flow. A rabbit was inoculated intravenously with 1 c.cm. of toxin, obtained by growing a smooth strain of the organism in beef broth for one month at 37° C. The leucocyte count 1 hour and 5 hours after inoculation was lower than

previously, but 24 hours later it had reached nearly seven times the resting figure : 80 per cent. of these cells were polymorphs. No agglutinins or precipitins were present. The animal lost 120 gm. weight, and died 48 hours after inoculation.

A second rabbit was inoculated similarly with 2 c.cm. toxin, obtained from a month's growth of a rough strain. The leucocyte count rose to five times the resting level ; half of these cells were polymorphs. No agglutinins or precipitins were present. The animal lost 70 gm. weight, and died in 48 hours.

A third animal received 0·5 c.cm. of toxin from a 10-day growth of a smooth strain. The leucocyte count rose to double the resting level in 24 hours and then dropped, to show a slight leucopenia in 72 hours. No precipitins were present, but agglutination up to $\frac{1}{100}$ was observed. The animal became completely paralysed in a week, and died.

Somewhat similar blood-changes were observed in the non-fatal experimental intoxications of rabbits.

Immune substances were formed in rabbits inoculated with 1 month, 10 day, and 1 day toxin, provided the degree of toxicity was slight. A reaction of 1 in 1,000 to a Shiga antigen may develop from one injection of Shiga toxin. Control experiments were made by injecting rabbits with sterile beef broth, which was the medium used for the preparation of the filtered antigens, and with filtered beef broth in which either *S. aureus, B. paratyphosus* B, or a hæmolytic strain of *B. coli* had been cultivated for one month at 37° C. The antigens were prepared by the same technique in every detail as was used for the Shiga antigens, and 1 c.cm. was injected intravenously in each case. All these beef broth filtrates produced much less effect than the Shiga filtrates on the total white cells and total polymorphs.

The tissues were examined from rabbits which had died from Shiga toxæmia, and also from control rabbits which had recovered, but were ultimately killed by bleeding from the axillary artery under anæsthesia. The tissues from rabbits which died from the Shiga bacillus and *S. aureus* injections were also used for control purposes. Death occurred among the rabbits inoculated with Shiga toxin in from 36 hours to 4 days, on the average in 48 hours. Liver, lungs, spleen, heart, and in a few instances kidneys, and great omentum were put into Kaiserling's fluid at the time of the post-mortem examination, which took place in most instances immediately after death. A summary of the results of the microscopical examination of the tissues is as follows :

Liver : Marked fatty change in liver cells. Cells swollen and outline indistinct. In some instances, polymorphs abundant in the liver sinuses ; in others, less obvious ; but in all instances they were readily seen between the liver cells.

Lungs : Polymorphs were abundant in the alveolar walls in some of the rabbits and less so in others ; in those instances in which they were abundant they gave a striking appearance to the lung tissue. The

alveolar walls were congested and small pulmonary hæmorrhages were present. One rabbit died in 48 hours from Shiga toxin ; the pulmonary vessels were thrombosed and the lungs were œdematous.

Heart and Spleen: The same polymorph reaction occurred in the heart muscle between the muscle cells, and in the splenic sinuses.

Rabbits which did not succumb to the Shiga toxin, but were killed within a few days from the time of inoculation, also showed a polymorph reaction in the tissues. Control rabbits injected with the live Shiga bacillus and *S. aureus* showed a similar polymorph reaction in the viscera.

Pathology in Man.

The Shiga bacillus enters the body by means of infected food, and within 12 hours the first symptoms may occur. The intestinal lesions are usually limited to the wall of the large intestine from the cæcum downwards, but in severe cases the last 1 to 2 feet of the ileum are similarly affected. The bacillus is found in the stools, in the deep layers of the mucosa of the intestinal walls, and in acute intestinal ulcers, and it may be isolated from the depths of old ulcers after the surface has been scraped. Shiga isolated the bacillus from the mesenteric glands, and it has been cultivated from the liver, but the number of bacilli obtained from these situations is small.

Blood infection plays little part in the spread of dysentery in the human subject, as shown by negative blood cultures and by the infrequency with which the Shiga bacillus has been obtained from the complications which occur in bacillary dysentery. Flexner (1907) considered that the dysentery bacillus produces an active poison which is the cause of the constitutional symptoms as well as the local intestinal lesions, and it is for this reason that intense inflammation of the intestines is associated with profound toxæmia. The acute lesions which are first seen on the elevations of the folds of the mucosa, and then at the lower levels, consist of swelling and necrosis of the mucous membrane, with formation of pseudo-membrane. The surrounding mucous membrane is hyperæmic, plum-coloured, and œdematous, often hæmorrhagic and covered with mucus of a glassy appearance which is frequently blood-stained. The changes in the mucosa consist of coagulative necrosis with exudation of fibrin and polymorphs : this exudate may entirely replace the true mucosa. The intestinal lesions, as in all inflammatory processes, depend on the severity of the infection, and the clinical symptoms and cytological characters of the stools depend upon the extent of these lesions. An accurate knowledge of the clinical symptoms of bacillary dysentery is a sure guide as to the extent of the pathological process, but only if the clinical observer has been fully trained in the pathology and bacteriology of the disease.

Acute dysenteric ulceration commences on the free edge of the valvulæ and runs transversely. The ulceration is ragged with undermined edges and the individual ulcers often intercommunicate. The œdema of the intestinal wall and the acute degeneration of the muscular coat of the

bowel add to the severity of the symptoms. In some cases of acute bacillary dysentery the degeneration in the muscular coat is very extensive. Unless complete recovery ensues various chronic inflammatory changes may persist and lead to fibrosis and strictures of the bowel. Mucus *retention cysts* may occur as a sequel to chronic bacillary dysentery beneath the scars of old ulcers. These may become infected with *B. coli* and according to Manson-Bahr (1919 20), septicæmia with pyæmic abscesses may terminate the case. Fletcher and Jepps (1924) have drawn attention to the presence of retention cysts in the mucosa in chronic dysentery, which appear as small papules with a minute central hole leading to small collections of muco-pus, from which the Shiga bacillus has been cultivated.

Hart (1918) has described subserous hæmorrhages and hyperæmia and œdema of the brain and pia mater which may be met with in acute bacillary dysentery, due to the toxin passing into the general circulation. Cutaneous hæmorrhages were uncommon. Acute ulceration of the duodenum is stated by Hart to have occurred in some cases.

One of the most serious effects of bacillary dysentery is the *dehydration of the tissues*, more especially when associated with vomiting. It leads to rapid wasting, cyanosis of the extremities, a rapid pulse of low tension, and muscular cramps. It is in these cases that a considerable leucocytosis may be met with.

Manson-Bahr (1921) like other writers on bacillary dysentery considers *dysenteric arthritis* a severe complication. The knee is most commonly affected, though ankle, hip, metacarpo-phalangeal, metatarsal, and tempero-maxillary joints have been affected. Arthritis generally supervenes during convalescence, or when the acute symptoms have subsided. The effusion into the joint takes place suddenly and is accompanied by pyrexia. The synovial fluid is straw-coloured and generally sterile on culture. Manson-Bahr refers to the work of Klein who has shown that the synovial fluid possesses considerable agglutinative power for the homologous organism: the titre, in fact, may be as great as that of the blood serum.

Some 25 cases of acute arthritis occurring in one or more of the large joints were investigated by my colleagues in the East during the war. In the majority of these cases the diagnosis of antiserum arthritis could be excluded. Conjunctivitis complicated the arthritis in some instances, but no investigation was made of the conjunctival exudate. The urine from four cases of arthritis complicating Shiga dysentery was examined by Elworthy (1918), but no dysentery bacilli were cultivated. The synovial fluid from the infected joints was examined bacteriologically in 10 cases of acute bacillary dysentery, of which 7 were proved Shiga infections. The fluid was very turbid owing to the abundance of leucocytes, and although no bacteria were seen in film preparations of the exudate, Elworthy (1918) cultivated the Shiga bacillus in one instance: from a large quantity of the joint exudate which he added to his media, four colonies of the Shiga bacillus were obtained. The joint fluid also contained

true Shiga agglutinins. This case is of considerable importance because of the isolation of the Shiga bacillus, as it is the only recorded case as far as I am aware. The joint exudate in the other cases was examined by exactly similar technique, but the fluid was sterile. Fluid from the affected joints of two cases was injected into the knee joints of two rabbits, also intraperitoneally, and, in one case, into the anterior chamber of the eye. No ill effects were observed, and no agglutinins had formed in the blood in three weeks.

Iridocyclitis may supervene in association with the arthritis, or independently during convalescence. It is stated to occur only in Shiga infections.

Bittorf (1918) found that 1 per cent. of cases of bacillary dysentery developed *neuritis* which usually started during convalescence or when the acute symptoms had subsided and was apparently excited by rapid loss of body weight. It was generally a sensory neuritis, as only one case of severe motor paralysis occurred. Other observers have also met with this complication. *Pyæmic symptoms* with recurring chills, due to multiple abscesses in the liver, may occasionally occur. Unilateral or bilateral, simple or suppurative *parotitis* may develop, but is very uncommon in my experience.

In bacillary dysentery death may be due to cardiac failure, but the severe dehydration of the tissues which occurs may lead to vaso-motor changes and failure of the circulation. Loss of weight may be very rapid ; in fact, some of the Shiga cases are so shrunken as to be devoid of subcutaneous tissues and to consist only of bones, skin and shrunken muscles. Fletcher and Jepps (1924) have shown that the average weight of healthy labourers in a certain district in the East is 8 stone, while the average weight of 105 of these labourers who died at the district hospital from bacillary dysentery was 5 stone 6 pounds.

Mechanism of the Spread of Infection.

Viability.

Schmidt, quoted by Flexner (1907) failed to isolate the dysentery bacillus from mixtures of earth and water, or milk exposed to the full degree of the cold of winter, but Pfuhl (1902) who allowed fæces containing dysentery bacilli mixed with earth to be exposed to a temperature of 1·5° C. to 15° C. for 101 days was able to recover the dysentery bacillus at the end of this period. Flexner (1907) states that dysentery bacilli survive in air-dried fæces for 12 days, and in soiled linens for 17 days. In water he found that they survived from 5 to 9 days, and in butter and cheese about the same time. Sunlight, according to Shiga, killed his bacillus in 30 minutes, while it has been stated that it will survive drying for 10 weeks or longer if not exposed to the direct sunlight.

Distribution.

In animals. Dold (1916) examined 7 sporting dogs at Shanghai suffering from diarrhœa, 4 of which were found to be infected with

dysentery bacilli. One dog which was 2 years old had 4 to 5 motions daily, passed blood for 2 weeks, and died from the disease. The Shiga bacillus was isolated from the fæces and blood before death, and the dog's serum agglutinated this organism. No autopsy was performed in this case. Dold and Fischer (1920) record an autopsy on a dog which died from dysentery. The Shiga bacillus was isolated from the intestinal mucosa, which showed inflammatory changes.

In carriers. Fletcher and Mackinnon (1919) investigated 935 dysentery convalescents and 847 patients convalescent from other infections such as enterica and trench fever. They found that 13 of the dysentery cases were carriers of *B. dysenteriæ* (Shiga). Each of these patients was a persistent carrier (three months or longer), and suffered from chronic dysentery with mental depression and was unfit for work. Together they were examined 469 times, and the bacillus was isolated on 207 occasions.

Convalescent cases of bacillary dysentery before discharge from hospital should be examined to see if they are able to carry out light duties and take ordinary food. Those who are 'cured' can lead an absolutely normal life in every detail. If, however, diarrhœa, with or without obvious mucus in the stools, occurs from any cause, then the stools should be examined bacteriologically, film preparations made of the intestinal mucus and serological examinations undertaken. Carriers of *B. dysenteriæ* (Shiga) are in my experience sick men : they may be very considerably below their normal weight and suffer from various nervous phenomena, and are at the mercy of their intestinal tracts. In some cases, Morgan's bacillus, *B. pyocyaneus*, *B. fæcalis alkaligenes*, and bacilli which ferment lactose slowly or not at all may be present in the fæces in abundance.

The importance of the presence of mucus in the stools of convalescent patients should be recognized so that a patient is not discharged from hospital whose stools contained mucus, even if only occasionally. By this means the risk of carriers being discharged from hospital would be slight. In my experience the Shiga bacillus isolated from cases of acute or chronic dysentery is always virulent when tested on animals and shows a high degree of toxicity. I have never met with an avirulent culture of *B. dysenteriæ* (Shiga).

Method of Transference to Man.

In the vast majority of cases, the organism of bacillary dysentery is found only in the fæces ; therefore, infection by direct contact is very difficult to prove. Medical officers and nurses working in dysentery wards have contracted the disease, but probably from food infected either by flies or by other agencies. For direct infection to occur it is necessary to contaminate the hands with the fæces of a patient or with bedding or clothes soiled with fæces, and then infection must occur from the grossest negligence. Davidson (1907) states that direct contagion in dysentery is

practically restricted to cases in which an enema syringe used for a dysenteric patient is employed for another patient without efficient sterilization.

Food may possibly become infected in hot countries by dust or from uncooked vegetables directly contaminated with human excreta. It is well to remember that bacillary dysentery is a disease of hot countries in which sanitation is defective, or non-existent, and is especially likely to assume epidemic form if the inhabitants are in a low state of health. Every case of bacillary dysentery is likely to be the cause of many more cases unless the sanitation is efficient, and in countries where dysenteric fæces are deposited on the open ground, direct infection of food and water may occur from infected dust. It is, however, in my opinion, the fly that is the main source of danger in transmitting the disease by contaminating the food.

Water.

Water polluted with infected fæces is considered to be one of the chief means by which dysentery is disseminated. The disease, therefore, is especially likely to occur when the water supply is derived from surface wells, or is insufficiently protected from contamination. Buchanan (1918) reported an outbreak of bacillary dysentery in an institution in the Eastern Counties which occurred in December, 1917, and January, 1918. It was stated that the infection was water-borne, and that the infecting agent was the Shiga bacillus, although this organism was not recovered from the water. It was, however, isolated from nine of the dysenteric patients. Buchanan also refers to some experiments of Captain Benjafield who isolated the Shiga bacillus for 10 successive days from well water contaminated with this bacillus.

In Macedonia, Dudgeon (1919) carried out experiments on the Shiga bacillus planted in river and aqueduct water under varying conditions, when the following conclusions emerged. (1) The Shiga bacillus can be recovered from stored sterile water for a considerable period, which amounted to 576 hours in one of the experiments. (2) The organism will live and multiply in stored water, but especially in water stored at a low temperature. (3) The bacillus retains its cultural and agglutinative properties when stored in water. (4) These results emphasize the danger of storing ' sterile ' water without adequate protection from infected dust or other contamination. It appears to be a belief that water once sterilized remains fit for human consumption indefinitely.

Flies.

Smits (1915) concluded from his experience at Sumatra that the spread of infection by flies is important. He succeeded in isolating the Shiga bacillus from flies caught in the neighbourhood of dysentery patients and the dysentery barracks by extracting the whole fly in sterile water. Manson-Bahr (1919 20) considers that bacillary dysentery occurs most usually in epidemic form in late summer and early autumn, just at the time

the house-fly begins to multiply after the excessive heat. It is a disease of camps where the ground has become contaminated. He attributes an important role in the spread of the disease to the fly and found little evidence of contagion through food and water. He isolated the Shiga bacillus from the gut of flies caught in the desert two miles from the nearest camp.

Taylor (1919) has shown from his carefully planned experiments conducted in Macedonia during the Great War that there is a close relationship between the incidence of bacillary dysentery and the fly pestilence. He also made a large number of experiments on feeding flies and on the bacteriological examination of ' wild ' flies. Flies were fed on a milk culture of the Shiga bacillus and kept in separate cages. Every 12 hours the fæces of each fly was examined until death. The bacillus was isolated from 7 flies out of a total of 71 and in 6 instances in which the organism was recovered the time between feeding and plating was 12 hours. Forty-four flies were fed on a culture of the Shiga bacillus ; in 6 of the flies killed in less than 24 hours, out of a total of 14, the bacillus was recovered from the fæces, and in 1 out of 15 at 24 hours from the time of feeding. The Shiga bacillus was not recovered at a subsequent period. Taylor examined 1,670 ' wild ' flies which he trapped in the wards, kitchens, latrines, &c., of two general hospitals. Fifteen hundred of these flies were examined by the ' walking ' method, and 170 by plating the fæces. One thousand two hundred and forty were examined at a period when the incidence of dysentery was high and the flies were very active. *B. dysenteriæ* (Shiga) was not recovered, but from one fly a bacillus which was culturally identical with the Shiga bacillus, but inagglutinable, was isolated. Three hundred and thirty active flies captured in one hospital at a time when the incidence of dysentery and the fly incidence were high were examined as follows : 160 by the walking method and the fæces of 170. Typical *B. dysenteriæ* (Shiga) was isolated by the walking method from a fly captured in the hospital kitchen.

Natural and Naturally Acquired Resistance.

Fletcher and Jepps (1924), in support of their view that dysentery is a disease of poverty, have classified the patients admitted to their wards in Kuala Lumpur as follows : (1) Europeans. Mortality negligible. (2) Eurasians. Well-to-do Asiatics. Mortality 2 to 3 per cent. (3) Native labourers, paupers and vagrants. Mortality 25 per cent. There is no question that bacillary dysentery is a much more serious disease in the ill-nourished, exhausted, and improperly fed than among those who have become infected while in a good state of health. It is for this reason that it is such a serious disease in times of war and during epidemics.

Patients who have recovered from an acute attack of Shiga dysentery may show a high titre of agglutinins in the blood-serum. This fact does not justify the assumption that true immunity to the infection is present, as a relapse may occur in spite of the presence of the immune substances.

The only true protection is a healthy intestinal wall. This leads us to a question which has often been debated. Does one attack of Shiga dysentery afford protection against future attacks? Some experienced workers on the subject of dysentery believe that a subsequent attack of Shiga dysentery is really a recurrence of the first attack. If a period of perfect health has existed for some two or three months so that an individual can eat and drink without restriction, is able to take full physical exercise in cold and damp weather, and is up to full standard of weight, then if an attack of Shiga dysentery occurs, it is most probably a fresh infection, and not a relapse. There is abundant evidence that Flexner infections occur in individuals who have had Shiga dysentery and the converse is equally true, but this fact is really beside the question because the Shiga bacillus and the Flexner bacillus are two distinct organisms. Streit (1918) considers, chiefly from his perusal of the literature, that bacillary dysentery is generally followed by lasting immunity, which is strictly specific for each dysentery bacillus.

Practical Diagnosis.

Detection of the Organism in Stools.

To obtain a culture of the Shiga bacillus in acute bacillary dysentery a portion of mucus should be selected from the stool and plated on one of the agar media, preferably litmus lactose agar. When the mucus is intimately mixed with the fæces, it can be separated by spreading the material lightly on a sterile unglazed porcelain tile. As soon as partial drying has occurred, which is within a few minutes, the mucus can be picked off and plated out on the appropriate media. A liquid or semi-liquid motion should be spread on a sterile unglazed porcelain tile until it is relatively dry, and then transferred to a second tile to complete the drying, according to Dudgeon's technique, which is described in detail by Wordley (1921). The powdered fæces is then added to the agar medium and spread in the usual manner. Opinions differ as to the best of the agar media. Fletcher and Jepps (1924) recommend eosin methylene blue agar as a more satisfactory medium for the tropics than Endo's. They consider that trypsinized broth agar offers no advantage over ordinary lemco or marmite agar; a view with which I am in agreement. Other workers prefer MacConkey's neutral red lactose agar, brome-cresol lactose agar or phenol red lactose agar, but I prefer litmus lactose agar to any other medium. It is readily prepared, the Shiga bacillus grows freely on it, and it is free from inhibitory substances; but it must have the correct reaction, which is pH 7·4 or 7·5. Stitt (1927) also considers litmus lactose agar to be the most satisfactory medium for the purpose. It is necessary, whatever agar medium is employed, that the surface of the medium should be free from moisture.

Selected colonies from the agar plate must be examined culturally in the various media which are employed for this purpose, and tested serologically with immune Shiga serum.

The acid-agglutination test. During the war, Michaelis (1915 and 1917) recommended for the identification of dysentery bacilli the acid-agglutination method, which, if accurate, would have been of the utmost value because of its simplicity. In this method solutions of normal sodium hydrate and normal acetic acid are added to bacillary emulsions in definite but varying quantities. Six tubes arranged in series are required for the reaction, each tube containing varying quantities of the acid-alkali mixture. According to Michaelis, dysentery bacilli *never* show agglutination by his method, and even if a trace of blood serum is added to the acid-alkali mixture no agglutination of dysentery bacilli occurs. But Murray (1918) concluded that the reaction was of no value in the separation of dysentery from other bacilli. Dudgeon (1919), from experiments in Macedonia, came to the same conclusion.

Examination of Blood and Urine.

Blood cultures were made on 145 cases of acute bacillary dysentery by my co-workers in Macedonia during the war, but the Shiga bacillus was not recovered from the blood-stream. The examination of the blood was made during the acute stages of the disease by taking 5 to 20 c.cm. of blood by vein puncture and adding this to tubes of glucose broth, distilled water, and 2 per cent. bile salt in distilled water. Our failure to isolate the Shiga bacillus from the blood stream in acute Shiga dysentery is in agreement with the experience of most workers on this subject, and only a few instances have been reported in which the bacillus has been isolated. Darling and Bates (1912) published a case of acute Shiga dysentery from which they claim to have isolated the Shiga bacillus from the blood. The incident is so uncommon that a brief extract of the case is worth recording. A patient was ill with acute dysentery ; suffering from severe intestinal hæmorrhage and frequently stools of blood, mucus and pus. The temperature was 101°. Leucocytes, 27,500 per c.mm. A blood culture was made by taking 4 c.cm. of blood into a bile glycerin peptone medium on the sixth day of the disease. At the end of 24 hours at 37° C., an abundant growth of a bacillus was obtained, which proved culturally to be the Shiga bacillus, although litmus milk remained permanently acid. The bacillus agglutinated to the full titre of a mixed Flexner-Shiga serum. The patient died on the tenth day of the disease.

I know of no instance in which the Shiga bacillus has been recovered from the urine of a patient suffering from bacilluria or any infection of the urinary tract.

Enrichment.

It is a fact well known to bacteriologists who have studied this disease in sub-tropical and tropical countries that a high percentage of positive findings can be obtained only if the samples of stools are received in the laboratories in the fresh state. If the specimens consist of blood and mucus, an interval of a few hours is possible between the time the sample is collected from the patient and the bacteriological examination, but if

fæces are present with blood and mucus it is essential to avoid delay. When many hours must elapse between the collection of the specimen and its arrival in the laboratory, it is necessary to employ some device which may counteract the changes that occur in stools consisting of blood and mucus mixed with fæces, or in diarrhœic stools. Various methods devised for this purpose were tested in the laboratories in Macedonia during the war by myself and my colleagues. The work of Nicholls (1917) who directed attention to the susceptibility of the *Vibrio choleræ* to acids, and of the dysentery group to acids and alkalies, induced me to investigate the action of acids and alkalies on dysentery bacilli. It was found, after numerous experiments, that lactic acid was markedly inhibitory to the growth of dysentery bacilli, especially the Shiga bacillus, which, however, grows in media containing even 6 per cent. of normal sodium hydroxide. Its vitality, inhibited by lactic acid, may be restored by alkali. These observations suggested that the addition of alkali to a dysenteric stool would facilitate the isolation of dysentery bacilli, and it was found that if the bacteriological examination was likely to be delayed, the intimate admixture of an equal volume of 3 per cent. normal sodium hydroxide with the freshly collected stool was of practical value (Dudgeon, 1919).

Fletcher and Jepps (1924) employed the solution advocated by Teague and Clurman (1916) for mixing with the stools. It is claimed that the Shiga bacillus, which is so difficult or even impossible to isolate from stools which have to be stored for many hours, can be readily cultivated when the specimen is mixed with this solution. In this method one part of fæces is mixed with two parts of the fluid, which consists of 30 per cent. of glycerin in 0·9 per cent. sodium chloride. It was found that the Shiga bacillus could be cultivated in every instance from fæces left in contact with this solution for 6, 7 and 8 days. For control purposes, 15 samples of fæces were obtained from 5 patients suffering from acute bacillary dysentery due to the Shiga bacillus. The specimens were stored at room temperature in glass vaseline pots with screw tops, and were examined every day. In 10 out of 15 samples, the Shiga bacillus could no longer be cultivated within 24 hours of storage, in four instances the bacilli were isolated on the second day, and in one instance, on the third day. Teague and Clurman (1916) found that in seven specimens of fæces obtained from a man suffering from acute Shiga dysentery, the Shiga bacillus survived much longer when the fæces were emulsified in the glycerin mixture than in the untreated specimens. In two cases the bacilli died out as quickly in the emulsion as in the control, but in the other five instances they persisted in the emulsion for 29, 17, 13, 9 and 7 days respectively, which is considerably longer than the maximum period of 3 days in the untreated fæces. It was found that even in specimens stored in the glycerin solution there was a considerable fall in the percentage of dysentery bacilli during the first 36 hours.

These experiments were carried out in England with a laboratory temperature of 15° C., and they have been repeated at Kuala Lumpur,

where the temperature is approximately 30° C., and the results have been the same. In Shiga dysentery no organism of the Shiga type was isolated from samples which were more than 24 hours old without addition of glycerin, except in one instance where they were found as late as the fourth day. When fresh samples were mixed with glycerin the dysentery bacilli survived for a much longer period—the thirteenth day in one specimen and the sixteenth day in another. In a small epidemic of dysentery which occurred in a remote corner of the Malay States specimens of fæces consisting of blood and muco-pus were mixed with the solution and forwarded to the laboratory. They were 6, 7 and 8 days old when they arrived, yet the Shiga bacillus was isolated from all three.

Cytological Examination.

The naked eye and microscopical characteristics of the stools in the various stages of bacillary dysentery have been studied by numerous workers on the subject, but Graham (1918) has shown that a definite picture of the pathological changes in the wall of the intestine is obtained by this means.

During the first 24 hours in severe cases of bacillary dysentery the material passed *per rectum* consists of relatively clear mucus streaked with blood or containing globules of red cells. This is due to the intense congestion of the mucous membrane of the large intestine, which secretes a large quantity of mucus. Film preparations made at this period reveal numerous red blood cells, white cells well preserved and a few epithelial cells.

During the next 24 hours the evacuation consists of bright red blood, semi-opaque mucus and a clear fluid. Microscopically there are a large number of red cells, abundance of pus cells which may be clumped, epithelial and endothelial cells. From the second to the fourth day the stools consist of semi-opaque mucus, little blood and opaque fluid. Bright red blood is no longer a predominant feature, the fluid now becomes opaque and microscopically it consists almost entirely of pus cells. About the fifth day, in a severe case little or no blood is to be seen macroscopically and the mucus appears purulent. Microscopically about 90 per cent. of the cells are pus cells, the remaining 10 per cent. consist of a few red cells, lymphocytes, endothelial, epithelial and plasma cells. At this period, coagulation necrosis and ulceration of the mucosa are taking place. Quite often at this stage or a little earlier one encounters blood and clear mucus in the evacuations, which indicates a bacillary dysentery of one day's duration, due to extension of the disease to a fresh area. After the fifth day, depending upon the severity of the case, fæcal matter reappears in the stool. ' Tags of mucus ', which may be present at this period or later, consist of degenerated pus cells, endothelial and epithelial cells. In the fæcal portion of the stool pus cells occur singly or in small groups of three or four.

In mild bacillary dysentery macroscopic evidence of blood disappears from the motion in a couple of days and purulent mucus is not seen. Semi-purulent mucus is found until the fifth day, and at the end of a week a fæcal stool contains tags of mucus.

Immune Substances in the Intestinal Exudate.

Davies (1922) has attempted to show that the intestinal exudate in bacillary dysentery is especially suitable for the examination of antibodies (agglutinins). He believed that from the results obtained by this method it would be possible to commence serum treatment at a very early period. He found that the agglutinins for dysentery bacilli were present in the intestinal exudate in the earliest period of infection, although they could not be demonstrated in the blood. Forty stools were specially investigated and 15 of these were in the first week of bacillary dysentery. The fluid portion of the stools was employed, and in those cases in which none was present the stool was shaken with normal saline and this was used for the serological investigation. Of 24 cases of the first group, the Shiga bacillus was isolated in 15, and in 18, Shiga agglutinins were demonstrated by Garrow's method, the titre varying from 1 in 10 to 1 in 80. In the second group of 10 cases, the Shiga bacillus was isolated in 4 and the Flexner bacillus in 1. Agglutinins were demonstrated in 4 of the cases in saline washing. By this method of examination of the intestinal exudate, Davies states that he was able to show the nature of the infection in from 12 to 16 minutes. It would require a considerable amount of work on cases of bacillary dysentery to prove that this method is strictly accurate. We are able to distinguish between acute bacillary and amœbic dysentery in a few minutes by a cytological investigation, but Davies claims that by his method the nature of the bacterial infection is proved, so that the right serum can be administered.

The Agglutination Test of the Blood-Serum.

Shiga antigens are prepared by growing the bacillus in veal broth or on agar and subculturing twice daily for 10 subcultures. If agar antigens are to be employed, then the last subculture is made on a large flask of agar, which is incubated at 37° C. for 24 hours, and the antigen is washed off with sterile normal saline containing 0·1 per cent. of formalin, filtered through sterile linen, and stored in the ice safe until it is sterile. The veal broth antigen is killed with formalin and filtered in the same way. Either antigen is then reduced in strength to an arbitrary number of 500 million bacilli per c.cm. as estimated by Brown's tubes. Small conical tubes containing the antigen and dilutions of antiserum are partially immersed in a water bath at 52° C. for four hours. A temperature of 52° C. is better than 37° C. because the reaction develops earlier and is more conclusive both as regards intensity and end-point. The reactions are read with a hand lens focussed on the tube placed in a suitable light against a black background, but the reactions should be at room temperature for 10 minutes before the reading is taken. Each antigen

is titrated against a known Shiga antiserum so that the agglutinability of the antigen is controlled. The results are expressed in the form of a fraction—patient's serum over immune rabbit serum.

Ritchie (1916) studied the agglutinating value of the sera of normal persons for the Shiga bacillus. He used live emulsions obtained by washing off the growth from a 24-hours' agar culture with normal saline. The reactions were carried out in an air incubator at 37° C. for two hours, and were recorded by microscopical examinations. Seven hundred and ninety-two normal persons were examined by this method, and as a result Ritchie considered that complete agglutination in a dilution of 1 in 64 and over should be regarded as diagnostic of a Shiga infection. The 'normals' were carefully selected: men and women who had not been abroad and had not suffered from dysentery. I have no experience of Ritchie's method, but his limit for the normal is much higher than mine.

Martin, Hartley and Williams (1918) employed Dreyer's method for the study of agglutination in the diagnosis of dysentery. A positive reaction was obtained in all their 13 cases of Shiga dysentery, and they consider that the agglutination reaction is of great value in the diagnosis of a Shiga infection.

This is also indicated by my experience in Macedonia. The sera from 177 cases of acute Shiga dysentery were examined. The earliest period in which an examination of the blood was made was the fourth day of the acute disease. Seventy-seven cases out of a total of 177 failed to react with a dilution of 1 in 25, but in 73 of these cases the blood was tested before the tenth day of the disease. Positive reactions varying from 1 in 25 to 1 in 200 were most frequently met with, and all reactions greater than 1 in 200 occurred in the second or third week of the disease. The highest reading recorded, 1 in 480, was obtained on the fourteenth day of the disease. Several cases gave a history of a previous attack of dysentery, and for this reason the possibility of pre-existing agglutinins in the sera of patients who give strong reactions must not be lost sight of.

Every sample of serum examined for Shiga agglutinins was tested also for the presence of Flexner agglutinins. Two or three Flexner antigens prepared from different Flexner strains were always used. The total number of Shiga cases which gave a Flexner reaction of over 1 in 100 was 112. In 50 cases the reaction was between 100 and 200, in 22 between 200 and 300, in 22 between 300 and 400, and in 18 cases between 400 and 800. The large number of Shiga cases with a high Flexner reaction is of interest.

Speares and Debono (1919) as a result of their investigations came to the following conclusions: Shiga agglutinins may appear about the tenth day of the disease and a rising agglutination may occur, Shiga infections raise the Flexner agglutinin content of the blood apart from a Flexner infection. These results were obtained especially with one Flexner strain 'Gallipoli' (Oxford V.) while with two other Flexner strains no reaction occurred except in low dilutions.

Lancelin and Rideau (1918) refer to, but disagree with, what is known as the 'Martini-Lentz law', by which it is understood that patients infected with the Flexner bacillus develop Flexner agglutinins, but no Shiga, while the converse is also believed to hold good. They cite 15 cases of Shiga dysentery in which Flexner agglutinins were present.

It might be suggested that the majority of our Shiga cases were infected with both Shiga and Flexner bacilli, but the absence of the Flexner bacillus from the bacteriological findings, and the fact that a double infection is in our experience uncommon, does not support this view, more especially as the Flexner agglutinins were present in 112 cases of acute Shiga dysentery.

In 15 cases of dysentery due to the para-Shiga + or — group, in which the blood was examined, there was no reaction for the true Shiga bacillus.

I consider that a reaction of 1 in 40 is a positive indication of a Shiga infection if a formolized antigen is employed in the manner referred to, and that a reaction of 1 in 25 is strongly suggestive. In such cases the blood should be re-examined about one week later, as a rising reaction may be detected and will establish the diagnosis. A return of the pyrexia or an increased pyrexia is generally accompanied by a rise in the agglutinins.

Immunization and Specific Therapy.

Immunization of Animals.

The immunization of animals against Shiga infection has been investigated by various workers, and the difficulties are fully appreciated. Animals are very sensitive to live cultures, vaccines and filtered broth cultures of the Shiga bacillus, all of which are very toxic. For this reason the process of immunization must be carried on with great care with small doses of vaccines at the outset, as it is some considerable time before animals develop a high degree of immunity to maximum doses of live *B. dysenteriæ* (Shiga).

Thomson (1916) studied various methods of preparing vaccines for immunizing animals against the Shiga bacillus. The vaccines which he used were as follows:

(1) Heat-killed vaccines (56° C. for one hour), and heat-killed vaccines treated with normal horse serum by the method advocated by Broughton Alcock (1914), or with dilute specific Shiga agglutinating serum.

(2) Unheated carbolized vaccines. These included carbolized vaccines not sensitized, and carbolized vaccines which had been treated with immune Shiga rabbit serum.

Rabbits, thus treated, were tested for immunity by injecting live Shiga bacilli intravenously. The injection of 200 million live bacilli, 24 hours old, was found to be almost invariably fatal to the normal healthy full-grown rabbit. If a vaccinated rabbit had acquired a reasonable degree of immunity, it would tolerate a dose of 1,000 million.

Thomson found from his investigations that the toxicity of vaccines was best determined by the result of the first injection into rabbits.

Toxic vaccines either killed the animals or produced a considerable loss of weight. Bowel symptoms were most severe after the injection of heat-killed vaccines which had been treated with diluted specific Shiga serum before they were heated. Symptoms suggesting a lesion of the central nervous system were on the whole most severe after serum-treated vaccines, but they produced less loss of weight than the untreated vaccines. Thomson considered that the first dose was the critical one with each type of vaccine ; even a dose of 50 million might prove fatal. The second dose should not be more than twice the first. Heat-killed Shiga vaccine should be avoided, as heat does not reduce the toxicity, and it destroys that part of the antigen which produces the complement-fixing immune body. Vaccines sterilized by weak carbolic acid do not lose any of their immunizing value : in fact, they give a better immunity. Immunity, as estimated by the power to withstand lethal doses of Shiga bacilli injected intravenously, was obtained with serum-treated and untreated vaccine equally ; but its duration was not determined in either case.

Dean and Adamson (1916) recommended eusol as a means of reducing the toxicity of Shiga vaccines. Their method is fully described later (immunization of man).

Many observers have studied various methods which have been employed to reduce the toxicity of the Shiga bacillus, but the recent work of Torikata and Fujimoto (1927), if fully confirmed, is the most important contribution to the subject. They have shown that boiling a suspension of the Shiga bacillus at 100° C. for 20 minutes reduced its toxicity for rabbits to about 1/100. Rabbits immunized with broth cultures of the Shiga bacillus, or saline suspensions of agar cultures boiled for 20 minutes acquired marked resistance against living bacilli or the toxins of the Shiga bacillus, and the blood of the inoculated rabbit showed bactericidal and antitoxic substances. If the bacterial bodies are removed from the Shiga emulsions after they have been boiled for 20 minutes and then rabbits are immunized with the extract, immune bodies are found in the sera. The sera of rabbits which had been immunized with a filtrate of the Shiga bacillus boiled for 1 *hour*, contained bactericidal and antitoxic substances. A comparison of the bactericidal power of therapeutic anti-Shiga horse sera, and anti-Shiga rabbit sera, obtained by immunizing rabbits with cultures of the Shiga bacillus boiled for 20 minutes, was in favour of the rabbit serum.

Immunization of Man.

Prophylactic vaccines. The great objection to the employment of Shiga vaccine is the serious local and general reaction which occurs. The local reaction is generally so severe from a dose of 200 million bacilli that this vaccine cannot be employed as a prophylactic measure.

Dean and Adamson (1916) introduced a method for the preparation of a non-toxic Shiga vaccine by treatment with solutions of eusol. The smallest concentration necessary to make the vaccine non-toxic was

1 in 1,000. Rabbits immunized with a eusol vaccine could withstand 10 lethal doses of an ordinary Shiga vaccine. Agglutinins did not develop, or were small in amount. They obtained similar results with hydrogen peroxide. Three persons inoculated with eusol Shiga vaccines showed no constitutional symptoms, and the local reaction was no greater than with a typhoid vaccine. The vaccine recommended consisted of 200 million Shiga bacilli per c.cm. killed by heating at 58 to 60° C. for one hour, then mixed with an equal quantity of freshly prepared eusol in normal saline, and kept at room temperature for 24 hours. One c.cm. of this vaccine, which contains 100 million bacilli in 1 : 1,000 eusol, should be injected subcutaneously for the first dose. The second dose, 10 days later, should be 400 million ; and the third dose, 200 million bacilli without eusol. Bacilli killed by heat may subsequently be required to produce a satisfactory immunity.

My experience with eusol-treated Shiga vaccines which had been stored for some time was not as satisfactory as these authors have suggested.

Vincent (1921) states that 2,175 people were inoculated with his polyvalent anti-dysenteric vaccine in doses of 500 to 750 million bacilli, with the result of a rapid decline in an epidemic of Shiga dysentery. Immunization occurs 5 to 6 days after the inoculation. In the 2,175 people inoculated, 33 cases of dysentery occurred, but these developed 4 days after inoculation. The sick rate was 16 per 1,000 among the vaccinated subjects and 228 per 1,000 among the non-vaccinated. Vincent's latest vaccine contains 2,000 million bacilli per c.cm., and is made from 8 strains of the Shiga bacillus, 5 of the Flexner, 1 of Strong and 3 of Hiss, and is given in doses of 2,000 million (see also under Flexner-group active immunization, p. 241).

Sensitized vaccine. Shiga, quoted by Murray (1918), vaccinated 10,000 Japanese in 1900 with a mixture of dysentery bacilli and anti-dysenteric serum, thereby lowering the mortality, but failing to reduce the general incidence of the disease. In the village of Koai, however, he succeeded in lowering the incidence from 28 per month to nil.

Broughton-Alcock (1914) immunized over 200 persons with a serum-treated Shiga vaccine and found that only a slight local and general reaction occurred, but no agglutinins were formed in the blood of the inoculated patients, and no immune body which would fix complement. The method employed for the preparation of this vaccine was as follows : A 24-hours' culture of the Shiga bacillus, grown on peptone agar, was washed off in normal saline, and then the emulsion was centrifugalized. The deposit so obtained was mixed with 2 c.cm. of normal saline and heated at 56° C. for one hour ; the bacilli were counted, and the bacillary emulsion was added to 20 c.cm. of heated normal pooled human serum or to 10 c.cm. of heated normal horse serum. The bacilli and sera were allowed to remain in contact overnight, but when human serum was used, the mixture was left in the ice safe. The bacillary emulsion was then centrifugalized and twice washed in saline, and the final bacillary deposit

taken up in normal saline, so that 1 c.cm. contained 350 million bacilli. Broughton-Alcock recommends four simultaneous subcutaneous doses of 0·25 c.cm., followed 8 or 9 days later by four doses of 0·5 c.cm. into the opposite side. Similar doses may be given later.

Boehncke (1917) introduced a toxin-antitoxin prophylactic dysentery vaccine, which he called ' Dysbakta '. This vaccine was used on a large scale in Germany and elsewhere during the war. Bürgers (1918) employed it and found that headache, malaise and nausea occurred in 9·5 per cent. after the first injection of 1 c.cm., and in 8 per cent. after the second injection of 2 c.cm. about six days later. The ratio of the incidence of dysentery among inoculated and uninoculated in the same district under the same conditions was 1 to 3·33; the mortality among the inoculated was 0, and among the non-inoculated it was 1·9. Schelenz (1918) gave Dysbakta to 1,300 civilians and 545 soldiers during an epidemic of dysentery in the East, in doses of 0·5, 1·0 and 1·5 c.cm. at intervals of five days. The epidemic subsided four weeks after the prophylactic injections were instituted. Bischoff (1918) supervised the inoculation of 15,000 persons with Dysbakta in doses of 0·5, 1·0 and 1·5 c.cm., given at intervals of five days. Only 1·65 per cent. showed a rise of temperature above 101·2 F., and in 86·25 per cent. no reaction occurred. Local and general reactions subsided in 24 hours. He considers that the immunity probably lasts from 3 to 4 months.

Sero-vaccine. Graeme Gibson (1917) introduced a prophylactic dysentery vaccine which was tried during the war. Four broth flasks were inoculated with four strains of the Shiga bacillus and incubated at 37° C. overnight. The contents of these flasks were then transferred to Roux flasks containing trypsin agar, and incubated at 37° C. Next day the growth was washed off with normal saline. Washings were all mixed and then killed with 1 per cent. phenol. Flexner and Y vaccines were prepared in the same manner as the Shiga. The final vaccine contained 2,000 millions each of *B. dysenteriæ* (Shiga), *B. dysenteriæ* (Flexner) and *B. dysenteriæ* (Y).

Serum was obtained from the Lister Institute of such potency that when 0·1 c.cm. was mixed with 500 million killed bacilli of a toxic strain of the Shiga bacillus rabbits were protected against symptoms of toxæmia. It was desaturated with Flexner, Y and Shiga bacilli until all agglutinins were removed, and then tested for its anti-toxic power on rabbits, by injecting 0·25 c.cm. of the vaccine together with enough of a 1 in 20 dilution of the serum to protect the rabbit from symptoms of toxæmia. If the serum was found to be efficient it was diluted until 0·25 c.cm. of the dilution contained 0·1 c.cm. of absorbed serum. Vaccine and serum were then put up in twin phials. The protective inoculations were given as follows:—*First dose:* 0·25 c.cm. of vaccine and 0·25 c.cm. of diluted serum were mixed in a syringe and inoculated. *Second dose:* Seven days later. This was made up of 0·5 c.cm. of each. Sometimes a third dose, the same as the second, was employed. Graeme Gibson (1918) has given

some records of the use of this sero-vaccine in the prevention of dysentery. No ill effects occurred and the local reaction was such that the inoculated arm was fit for use in 36 hours. The case incidence in three units were as follows :

Unit	*Uninoculated*	*Inoculated*
A	7·6 per cent.	2·4 per cent.
B	3·4 ,,	0·2 ,,
C	3·0 ,,	0·4 ,,

This sero-vaccine produces no negative phase, but it is more difficult to produce immunity with a dysenteric vaccine than with other organisms of the intestinal tract. A period of 21 days after the second dose was required for full protection, but three doses are recommended. The length of immunity is probably from 4 to 6 months, as with protective cholera inoculation. Graeme Gibson does not recommend his vaccine for universal use, but that it should be employed in epidemics, or at the beginning of the summer in endemic dysenteric areas.

In Macedonia in 1918, Gibson's sero-vaccine was tried, with these results :

Non-inoculated		*Inoculated*	
Total	*Cases of Dysentery*	*Total*	*Cases of Dysentery*
406	31	249	8
437	15	386	0
630	19	232	1
29	1	43	1
594	1	237	4
2,096	67	1,147	14

The troops referred to had two inoculations of the sero-vaccine, and the local reactions were not as severe as with ordinary vaccines.

The results quoted show that a considerable measure of protection may be obtained by prophylactic vaccines, but there is still much work to be done in the production of a vaccine which will give efficient protection against Shiga and Flexner dysentery at the same time. Protection is required for at least one year, and the local reaction must be so slight that the working capacity of the individual is not reduced. When a really satisfactory prophylactic vaccine is available it should be employed for all those who may run any possible risk of an attack of dysentery.

Oil vaccine. Olitsky (1918) does not recommend sero-vaccines because the specific immune response is reduced, and the injection of horse serum is a disadvantage because of serum sensitization. He has prepared a suspension of dysentery bacilli in almond oil (sweet almonds) which acts passively, as the bacilli are absorbed slowly, and the immune reaction is not interfered with. The neutralization of the oil must be complete. As a result of the slow absorption of the bacilli from the oily suspension only

slight local and general reaction follows. He also experimented with lanolin, but with this the absorption of the bacilli is too slow which is a definite disadvantage.

He found that on the first day after injection, an area of erythema develops, which is not especially painful, and a slight general reaction. Then in 24 to 48 hours an area of induration appears, which gradually fades, so that complete absorption occurs in 1 to 3 weeks. Animals inoculated by this means were protected against lethal doses of the live organism or of the toxic products. He employed it for man in doses of 5,000 million in almond oil, then 25,000 million of Shiga and Flexner in equal parts.

Oral administration. This method we owe to the work of Besredka, who introduced bile-sensitized bacterial vaccines for the purpose. Opinions differ as to the value of oral administration for protection against bacillary dysentery, but there is a considerable body of opinion in its favour. Nicolle and Conseil (1922) claimed that protection was afforded in man when Shiga vaccines were given orally. Pascal (1924) refers to an epidemic of bacillary dysentery which occurred at an asylum in France in 1923 and threatened to break out again 1924. In 1923, there were 65 cases of dysentery among 256 inmates of the asylum, which gives a sick-rate of 22·72 per cent. At this period vaccine protection had not been employed. In 1924, out of 410 inmates of the asylum, 399 were vaccinated with Besredka's enterodysenteric vaccine; 3 cases occurred among the vaccinated and none among the 11 unvaccinated. This gives a sick-rate of 0·75 per cent. No reaction was recorded. During the course of immunization, two fresh cases of dysentery occurred. Troude (1925) claims great importance for Besredka's method of prophylactic immunization. He records that nearly 3,000 men were vaccinated by this method in the Rhine Army in 1923–4, but not a single case contracted dysentery. He refers to the fact that bacillary dysentery is endemic in the Ruhr district, and cases had occurred in the Army of Occupation. Klüchin and Wigodtschikoff (1925), however, did not consider that Besredka's method of oral vaccination in dysentery was of any practical value.

Alivisatos and Jovanovic (1926) immunized rabbits with oral doses of 70 mgm. of dysentery bacilli, which they found afforded protection against four minimum lethal doses of the bacilli, and also (for a short period) against lethal doses of toxin. Oral doses of 80 to 90 mgm. produced in many cases undesirable effects and even death. The writers state that this method immunizes human beings easily and quickly, and that the protection lasts for a ' dysentery season '.

Gauthier (1924) reports that, in the autumn of 1923, 30,000 anti-dysenteric vaccinations were given by the mouth, and that no case of dysentery occurred among the vaccinated. These vaccinations were given at four centres in Greece and Macedonia. Liquid polyvalent vaccines either of Shiga and Flexner, or of Shiga, Flexner, Y and Strong's bacilli were prepared in Athens at the Pasteur Institute and State Laboratory.

They contained 35,000 million bacilli per c.cm. in saline suspensions or in beef peptone. The results were compared with those obtained with a dysenteric vaccine prepared according to the method of Besredka. Little or no reaction occurred. The vaccines were given one hour before food. In May, 1923, a grave epidemic of dysentery occurred among the refugees. There were 22 cases with 3 deaths among a population of 700. Oral vaccination of the refugees at once arrested the epidemic.

Maitra and Basu (1926) made a preliminary study in India with 'Bilivaccines' for immunization of rabbits against dysentery. The results obtained were so encouraging that oral vaccination against dysentery was tried in certain selected jails in Bengal and Madras. They employed vaccines prepared as follows: (1) Bilivaccine-Shiga consisting of compressed tablets of desiccated bacterial bodies prepared by La Biothérapie of France according to the formula of Besredka. (2) Mixed and sterilized emulsions of dysentery bacilli, Shiga and Flexner, prepared in the Laboratory of Tropical Medicine, Calcutta, from strains of organisms isolated locally. Doses of 100,000 million dysentery bacilli were given to adults on an empty stomach for three consecutive days. No food was given by the mouth within two hours of ingestion of the vaccine.

Bacillary emulsions were used in two jails and Bilivaccine tablets in a third jail, and both emulsions and tablets in a fourth jail. One thousand one hundred and thirty-six prisoners were treated orally, but no ill-effect was recorded. The results were as follows:

	Bilivaccine.	Bacillary emulsions.
Total number *vaccinated*	509	627
Dysentery among vaccinated	11	18
Percentage of incidence	2·16	2·88
Total number *unvaccinated*	1,053	4,516
Dysentery among unvaccinated	47	237
Percentage of incidence	4·46	5·2

Therapeutic Injections.

Vaccines. I have never seen any benefit from Shiga or Flexner vaccines in the treatment of acute bacillary dysentery, but a Shiga vaccine in doses of 10 to 20 million at the commencement may prove of benefit for arthritic complication. In chronic Shiga dysentery vaccines should be tried in conjunction with other necessary treatment. The preliminary dosage is about 10 to 20 million bacilli, and the doses can be gradually increased as circumstances permit, every 5 to 7 days.

Antiserum (Shiga). One of the disadvantages of the serum treatment in acute bacillary dysentery is that so many of the antisera on the market are mixed Shiga and Flexner. It is often possible to know definitely

which form of bacillary dysentery is present within 24 hours of the examination of the stool by isolating the bacillus, or even in less time by the method advocated by Davies (1922). At the outset mixed antidysenteric serum can be employed, but at the earliest possible moment the specific serum should be administered. It is well to realize that the treatment of dysentery with antidysenteric serum must be combined with other treatment, or the value of the antiserum will be largely lost.

Antiserum (Shiga) is prepared from the horse and requires considerable care in its preparation owing to the toxicity of Shiga cultures and Shiga vaccines. This antiserum is without any doubt of considerable value in the treatment of acute bacillary dysentery. It is especially valuable for the profound toxæmia which is so often present. It should be given as soon as there is any evidence of toxæmia and in sufficient quantity, and if serum treatment is postponed until the patient is too exhausted to respond no benefit will ensue. The mortality of the great epidemic of bacillary dysentery in Japan in 1898 and 1899, which was studied by Shiga, varied from 28·5 to 37·9 per cent. among cases treated in the ordinary way, but in cases treated with his antiserum the mortality was reduced to 12 per cent.

Fletcher and Jepps (1924) consider that when antidysenteric serum is given early and in adequate doses it is a specific cure for bacillary dysentery. The effects of the serum appear promptly, the tenesmus is lessened and very soon disappears, and the stools are reduced in numbers within 24 hours. Graham (1918) points out that as the symptoms of bacillary dysentery are due to absorption of toxin it is essential to neutralize the toxæmia at the earliest possible moment. Dysenteric antitoxin is of low antitoxic value and therefore large doses of the serum are required, much larger than are usually given. In severe cases the serum should be given intravenously if possible : if not, by intramuscular injections, so as to obtain rapid absorption. Graham obtained his best results from intravenous injections of from 60 to 80 c.cm., of serum followed by 150 to 300 c.cm. of normal saline twice daily for the first two days, and once daily for the next two days. In very toxic cases, 5 per cent. glucose in distilled water should be substituted for saline. The effect of the antiserum is rapidly shown by loss of tenesmus, relief from the abdominal pain, reduction in the number of stools and gradual loss of the toxæmia. In mild and chronic cases of bacillary dysentery the serum treatment is of little value. Graham's results are well shown by the fact that in the treatment of 200 cases of which the majority did not commence serum treatment until after the third day of the disease and were having 20 or more blood and mucus stools per day, the death rate was 1 per cent. Great care must be taken in the prevention of anaphylaxis, more especially if the antiserum is administered intravenously. A very small dose of 0·25 c.cm. of serum should be given at the outset, and the remainder should be administered very slowly. A subcutaneous injection of atropine before serum treatment is of advantage.

Schittenhelm (1918) recommends antiserum for the treatment of mild cases of bacillary dysentery in which the acute stages have lasted longer than 3 or 4 days, and for all toxic and severe cases. The initial dose should be 60 to 80 c.cm. given subcutaneously or intramuscularly and further doses of 50, 40 and 30 c.cm. should be given for 3 to 4 days.

Neumann (1918) strongly recommends the serum of Shiga convalescents for the treatment of Shiga dysentery, or in other words true specific treatment. Thirty-five to forty c.cm. of this serum is injected subcutaneously. A rapid reduction in the toxæmia, a fall in temperature, and general improvement occurs, which in his opinion is far greater than with animal serum.

Antibacterial Measures.

(Against Dysentery Bacilli in General.)

Disinfection. Fæces should be protected from flies, and crude carbolic acid or Jeyes' fluid should be poured over the stools directly they are passed. Stools which are required for bacteriological, or other examinations, must be preserved in sterile or clean receptacles with well-fitting covers. The Shiga bacillus, when present in pure culture, is readily killed by antiseptics: 0·1 per cent. formalin will kill saline emulsions or broth cultures of the bacillus in 24 hours at 37° C., if the formalin is allowed to act in a closed receptacle, such as a glass bottle closed with a wax cork, or similar contrivance: 0·5 per cent. phenol will kill the bacillus in about three days in the ice safe, but in 24 to 48 hours at 37° C.

Sterilization of water. Water should be boiled before use and must be most efficiently protected against subsequent contamination. Ice should not come in contact with food or water in localities where bacillary dysentery occurs. Water can be filtered if an efficient filter is obtainable, or chlorinated.

Care of milk. Infection from drinking milk is possible if dirty utensils, fly contamination, unclean milkers or milkers who are dysentery carriers are permitted, or if stored milk is insufficiently protected. In tropical and sub-tropical countries milk must be boiled before use and then completely protected against fly, dust and other contamination. The danger from fly infection is so very great that it is impossible to be too careful. The flight of the fly from the latrine to the larder should be fully recognized by all who are responsible for the prevention of bacillary dysentery. The burning of *all* refuse and destruction of the flies' breeding ground is the ideal sanitary measure, although difficult to accomplish efficiently. The common breeding grounds of the fly are human excreta, manure, waste food, and general filth, provided it remains moist.

Food protection. All food must be protected by mosquito net covers, which must be kept in constant use and free from defects.

Nursing. Dysentery cases should be nursed in special wards set apart for them with efficient latrine accommodation. Skilled nurses with a thorough understanding of the means by which the disease is spread are desirable.

Preventive inoculation. Bacillary dysentery occurs as an epidemic disease if there is overcrowding, physical exhaustion of the population and defective sanitation. Preventive inoculation is advisable with a pure Shiga vaccine if the epidemic has been proved to be due entirely to this bacillus, otherwise a mixed Shiga and Flexner vaccine should be employed. Owing to the severe reaction which may occur from Shiga vaccine, as described above, it is advisable to attempt to immunize the population with a vaccine so prepared that the local and general reaction is compatible with normal life.

Carriers. As carriers are known to be one of the chief causes of the spread of the disease, or of the continuation of an epidemic, it is obvious that every effort should be made to safeguard the healthy population against them. There are both acute and chronic carriers, but the acute carriers of bacillary dysentery are not infrequently due to 'hastening' convalescence for various reasons on the part of Medical Officers. This error should be avoided. If a patient, believed to be convalescent from dysentery, suffers from attacks of diarrhœa, passes mucus, is unable to take ordinary food without having an intestinal upset, or exercise for a similar reason, he should not be permitted to leave hospital and mix with healthy population. Considerable stress has been placed on three negative bacteriological examinations of the stools before a patient is discharged as cured, but as long as mucus is present in the stools there is always the possibility of persistence of the infection—more especially if the mucus is associated with diarrhœa. In such cases a full diet, alcohol, or exercise may lead to an attack of diarrhœa with mucus in the stools and dysentery bacilli. Ulceration of the bowel may be detected by the sigmoidoscope, and the dysentery bacillus can be isolated from a scraping of the ulcer, although other methods may have failed. The blood of suspected Shiga carriers should be examined for the presence of Shiga agglutinins, and those with a reaction of 1 in 25 or over should be regarded with the greatest suspicion. Mucus in the stools should be plated on litmus lactose agar, or other medium if preferred, as soon as possible after it is passed.

The acute carrier will probably lose his infection with more prolonged medical treatment, but the chronic carrier may continue to pass dysentery bacilli for a considerable period, amounting to many months from the onset of the acute illness. Such cases should be isolated and dieted, and treatment with vaccines, medicinal measures and local applications to the infected bowel should be employed. Stock Shiga vaccines are suitable, because of the specificity of the organism. Wolf, quoted by Kolmer (1923) claims good results in the treatment of old chronic cases of dysentery with heated vaccines. He begins with a dose of one million bacilli and increases up to doses of 5 to 10,000 million subcutaneously. When the inoculations were given intravenously every four days, Wolf obtained still better results.

Schmitz's bacillus.
(B. ambiguus ; B. para-Shiga +.)

Schmitz (1917) investigated an outbreak of dysentery among Roumanian prisoners during the war. During these investigations, which were made in the early months of 1916, he isolated a bacillus from the stools which resembled the Shiga bacillus culturally, but formed indole and was serologically distinct. Before this, Remlinger and Dumas (1915) had described a bacillus isolated from two severe cases of dysentery, which formed indole, but otherwise resembled the Shiga bacillus culturally. This bacillus was inagglutinable with Shiga and Flexner serum and with the patients' serum. It was much more toxic for guinea-pigs than the 'Y' bacillus isolated in the same epidemic in the Argonne. This is probably the first record of dysentery associated with the bacillus subsequently described by Schmitz, and now known as Schmitz's bacillus. Stutzer (1923) claims to have recognized the bacillus before Schmitz published his results, and has named the organism *B. para-dysenteriæ x.*

In February, 1917, the late Captain Cecil Clarke sent me a culture supposed to be an inagglutinable strain of the Shiga bacillus, which he had isolated from the stools of a soldier suffering from acute dysentery in Macedonia. This organism was culturally similar to the Shiga bacillus, but it formed indole and was serologically distinct. I immunized rabbits with it and all subsequent strains which were sent to me from cases of acute, subacute and chronic dysentery in Macedonia were tested with this antiserum. Dudgeon and Urquhart (1919) described this organism, which corresponds to Schmitz's bacillus, as *B. para-Shiga* +, in contrast with another bacillus which was isolated later from cases of dysentery in Macedonia, and which they called *B. para-Shiga* —. This bacillus did not form indole and, therefore, was identical on cultural evidence with Shiga bacillus, but serologically these organisms are now referred to here as *B. para-Shiga* + or —. From February, 1917, until about June, 1918, 30 strains of the indole-forming bacillus and 11 strains of the indole negative bacillus were isolated from the stools in Macedonia and fully investigated by Dudgeon and Urquhart (1919).

Thomson and Mackie (1917) obtained both the indole-producing and the indole-negative bacillus from cases of dysentery in Egypt. Hirschbruch and Forthmann (1919) have reported the case of an attendant who accidentally swallowed a pure culture of the Schmitz bacillus and developed dysentery $2\frac{1}{2}$ days later with the bacillus in the stools.

Various workers differ as to their views on the part played by Schmitz's bacillus in the ætiology of dysentery in man. Schmitz regarded his bacillus as a cause of dysentery, while Andrewes (1918), who named it *B. ambiguus*, considered that there was no positive evidence in favour of this view. Manson-Bahr (1919–20) regards the Schmitz bacillus as a product of a stale dysentery stool or derived from the necrotic mucosa, a theory which is unsupported by any facts. Others have stated that they have isolated both the Schmitz bacillus and Flexner bacillus from

the same specimen of dysenteric stools. This observation, however, does not assist us in proving or disproving its pathogenicity for man.

An interesting point referred to by Taylor (1919) is worthy of mention. He fed flies on a stool of blood and mucus from a case of acute dysentery, in which 90 per cent. of the colonies were Shiga bacilli, yet this organism was not isolated from 15 fed flies, but the Schmitz bacillus was cultivated from 4.

I have made a few clinical records of some of the cases which occurred in Macedonia during the war. Of 9 cases from which Schmitz's bacillus was isolated from the stools, it was present in almost pure culture in 3; in 1 case it was stated to be abundant, and in the remainder it was the only dysentery bacillus isolated. In 8 cases out of the 9 the patients were passing blood and mucus, and from 1 patient the organism was obtained in almost pure culture from the ulcerated bowel. The blood-serum from one case agglutinated the same bacillus to $\frac{40}{800}$, and failed to agglutinate the Shiga bacillus. From two blood-and-mucus stools, from a stool consisting of mucus and from a semi-solid stool, *B. para-Shiga* — was isolated and cultivated. The clinical course and the bacteriological findings in these cases certainly suggest that Schmitz's bacillus is the cause of acute dysentery in man. The criticism that immune substances have not been demonstrated in the blood of patients from whom either of these bacilli have been isolated will not hold because of the result obtained on one case referred to by Dudgeon and Urquhart (1919).

Morphology, Cultivation and Biochemical Reactions.

B. para-Shiga + is a Gram-negative non-motile bacillus, about $0{\cdot}9\mu$ to $0{\cdot}3\mu$ in length, which stains well with the ordinary dyes, grows well on the ordinary media and forms colonies similar to the Shiga bacillus on litmus lactose agar. It does not form gas in any medium; it acidifies glucose and has no action on mannitol, lactose, saccharose and dulcitol. Four of our 30 strains acidified maltose. Andrewes (1918) found that 3 out of 13 strains which he examined fermented saccharose in 4 to 8 days, but none of our strains acidified it although they were incubated at 37° C. for 5 days as a minimum, and 10 days as the maximum. Every strain in my hands has acidified litmus milk without thickening or coagulation, and alkalinity subsequently developed in all but four of the strains.

Stutzer (1923) considers that Schmitz's bacillus always develops a spermatic odour, while Ornstein (1920) has stated that a spermatic odour is often present.

The indole reaction was examined for after five days' growth at 37° C. and found to be positive in every instance. Ehrlich's reagents were added to tubes of peptone water for testing the reaction.

In contrast with these cultural reactions of the Schmitz bacillus, the para-Shiga — bacillus described by Dudgeon and Urquhart (1919) is culturally similar to the true Shiga bacillus, except that the final alkalinity in milk did not develop with every strain.

Serological Characters.

Dudgeon and Urquhart were unable to agglutinate any of their strains of the bacillus of Schmitz or *B. para-Shiga* — with anti-Shiga serum prepared at the Lister Institute, R.A.M. College and their laboratories in Macedonia, and anti-Shiga agglutinins were not absorbed by any strain of these bacilli. Gehrmann (1918) has stated that it is necessary to heat the Schmitz strain to 100° C. for one hour before it becomes agglutinable, but it is difficult to understand on what evidence this statement is based.

Dudgeon and Urquhart immunized rabbits with both strains, and antisera were prepared. The Shiga agglutinins were not reduced in the slightest degree when Shiga serum was saturated with Schmitz's bacillus ; and in the same way the Schmitz' anti-serum was not reduced when saturated with the Shiga bacillus.

The agglutination reactions of five strains of the Schmitz bacillus (*B. para-Shiga* +) and three strains of the Shiga bacillus, with their corresponding antisera, and also a Flexner antiserum, were investigated. The Shiga bacillus was not agglutinated by either Schmitz or Flexner antisera. *B. para-Shiga* + was not agglutinated by Shiga or Flexner antisera. The saturation of Shiga antisera with Schmitz bacillus, or of Schmitz antisera with Shiga bacillus, did not reduce the titre of agglutination. Antisera prepared by immunizing rabbits with strains of *B. para-Shiga* — agglutinated every culture of that organism, but did not agglutinate the Shiga or Schmitz bacillus. The agglutinins were absorbed from the serum only by *B. para-Shiga* —.

Immunization of Rabbits.

The sera were prepared from rabbits by injections of the live organisms intravenously. In some cases intraperitoneal or subcutaneous injections appeared to give better results. The immunization of rabbits with either Schmitz's bacillus or *B. para-Shiga* — is in our experience much more difficult than with the true Shiga bacillus. We never succeeded in obtaining a high titre serum in rabbits. It may be necessary to employ massive doses of vaccines of these bacilli to immunize rabbits. Infinitely smaller doses of a heat-killed vaccine of the Shiga bacillus would prove fatal to rabbits in 3 to 4 days.

Pathogenicity for Animals.

Feeding experiments. Four young rabbits were fed with saline emulsions of 24-hours' agar cultures of the Schmitz bacillus. The organism was introduced into the mouth by means of a syringe, or mixed with the food. The number of bacteria employed on each occasion varied from 1,000 to 3,000 million. One rabbit was fed once ; one, twice ; and two, three times, at interval of four days ; but no evidence of dysentery developed.

Intestinal inoculation. Abdominal section was performed on three rabbits and from 1,500 to 3,000 million bacilli suspended in saline, from young agar cultures recently isolated from cases of acute dysentery,

were injected into the lumen of the gut. In the case of one rabbit abdominal section was repeated six days later and a similar procedure was adopted. No clinical evidence of dysentery developed, but one rabbit died four days after inoculation, and two acute intestinal ulcers were found. There is no question that rabbits are far more resistant to injections of the Schmitz bacillus or *B. para-Shiga*— than to the Shiga bacillus. It is of interest, however, that intestinal ulceration should have occurred from the injection of a culture of Schmitz bacillus into the lumen of the intestine.

The Schmitz bacillus has been recovered from cases of acute dysentery of every degree of severity, and from mild and chronic cases; it has been recovered in almost pure culture from the blood-and-mucus stools. Agglutinins have been demonstrated in the blood of one case of dysentery due to this bacillus. The argument which has been advanced that the bacillus which culturally resembles the Shiga bacillus, but is not agglutinated with anti-Shiga serum, will not absorb it, produces a specific antiserum in immunized rabbits and is much less toxic for animals than the true Shiga bacillus, is really a Shiga bacillus in an inagglutinable phase and not a distinct organism, appears to me unconvincing. On the other hand, it requires more evidence than is yet available to prove that it is a specific organism.

Several workers on dysentery have isolated inagglutinable strains of the Shiga bacillus, but the important point, as yet unsettled, is whether all their strains correspond serologically to the group which Dudgeon and Urquhart (1919) described which were agglutinated by a specific antiserum.

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(*For References not given here see Group References, p.* 182.)

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Bacillus dysenteriæ (Flexner) Subgroup.

By A. D. Gardner.

Synonyms: Pseudo-dysentery bacilli, groups A, B, C, D, F, G, H (Kruse); Eberthella paradysenteriæ, (Bergey); Flexner's, Hiss and Russell's 'Y', and Strong's bacilli.

Definition and Description.

A group of closely related bacilli which possess all the characters of the dysentery group (see p. 161), acidify mannitol but not lactose, and are serologically distinct from the other members of the group. Its characters are as follows :

Rod-shaped ; average size in mature agar-cultures, 0·5μ by 1·0 to 1·5μ ; aerobic (facultatively anaerobic) ; non-motile ; no flagella, spores nor capsules ; Gram-negative ; non-acid-fast ; gelatin not liquefied ; milk, slight evanescent acidity, no clot ; agar, semi-translucent, greyish colonies, more delicate than *B. coli ;* broth, the normal (' smooth ') growth shows uniform turbidity without pellicle ; acidification, without gas production, of glucose and mannitol, but not lactose. Action on other fermentable substances inconstant. Optimum temperature, 37° C. Indole production said to be variable, but usually positive.

Cultivation.

Flexner group bacilli need the same conditions for growth as Shiga's bacillus (q.v., p. 185). They are, in general, of rather more robust habit than the latter. They grow somewhat more profusely and are little inclined to autolysis. In culture media hydrogen-ion concentrations of from 5·6 to 8·3 permit rapid multiplication, but 7·0 to 7·6 may be considered as the most favourable reaction (Cohen and Clark, 1919). These bacilli adapt themselves readily to increasing alkalinity, up to a concentration of 5 c.cm. of a 10 per cent. soda solution in 100 c.cm. of neutral broth (Bernhardt, 1912). In the higher concentrations they grow in bizarre forms, but readily revert to normal when put back into media of pH 7·2 to 7·6. To acid, on the contrary, Flexner bacilli, like Shiga, are very sensitive. It appears that bile, which inhibits the growth of Shiga's bacillus, has no deleterious action on the Flexner group.

Biochemical Reactions.

Action on Proteins.

Growth in sugar-free media containing protein derivatives, e.g. peptone water, gives rise, normally, to a decreased hydrogen-ion concentration, and usually to the production of indole. Nitrites are formed from nitrates and a number of volatile fatty acids are produced.

Indole. The majority of those who have studied this question believe that the reaction is inconstant, in that one and the same strain may at one time produce indole, and at another time none ; and that two otherwise identical strains may give entirely different indole-reactions (Lentz, 1913 ; Murray, 1918 ; Andrewes and Inman, 1919). In this connection an observation of Cunningham and King (1917–18) is of interest. They found that some strains which gave a negative reaction in 1·5 per cent. peptone water produced indole in a 2·0 or 2·5 per cent. solution.

Thjøtta (1917) examined the question with considerable care, and came to the conclusion that practically all strains of his Group I and II of the mannitol fermenters (i.e. our whole Flexner group) produced some indole when offered the most favourable conditions. In attempting to discover what these conditions were, he found that a number of his strains which gave a negative reaction in peptone-water produced indole in nutrient broth. Proper control-tests of the uninoculated broth were done. This difference is probably to be explained by the more profuse growth which usually takes place in broth ; otherwise it would be hard to understand, since glucose, which is always present in broth, is known to inhibit indole production. It seems probable, then, that the unreliability of the indole reaction in this group is more due to deficiencies of technique than to a true intermittency of the bacillary function.

Cunningham and King (1917–18) found a strong positive correlation between the production of indole and the fermentation of dextrin, but Murray (1918) was unable to confirm it.

Volatile fatty acids. The production of these substances by dysentery bacilli grown for 48 hours in 1 per cent. Difco-peptone with 0·5 per cent. of sodium phosphate, with or without 1 per cent. of dextrose, under aerobic and anaerobic conditions, has been carefully measured by Zoeller and Clark (1921). They found a strikingly profuse formation of formic and acetic acids in the presence of glucose, whether oxygen was admitted or excluded. In the former case the pH of the medium changed from the initial 7·1 or 7·2 to about 5·0, in the latter to approximately 5·3. Cultures incubated anaerobically without glucose produced much smaller, though quite appreciable quantities of formic and acetic acids, and also some butyric acid ; the pH changing to about 6·5. Finally, aerobic growth in the peptone solution alone gave rise to small quantities of acetic and propionic acids, insufficient to balance the simultaneous decrease of pH caused by protein-fermentation, and there resulted a net lowering of pH to about 7·5. No difference was made out between the behaviour of the Shiga bacillus and that of the mannitol-fermenters. Practically the whole quantity of volatile acids was produced in the first 12 hours of growth, after which time there was neither increase nor decrease up to 60 hours of incubation. The authors remark that ' the enormous quantity of formic acid produced by these bacteria may play a significant part in the digestive disturbance and toxic symptoms accompanying their infection of the human intestinal tract '.

Action on Carbohydrates, &c.

The fermenting powers of the Flexner bacilli, in so far as they are of assistance in identification and differentiation from other members of the group, have already been mentioned (Dysentery Group, p. 164, and Table I). We need only consider here the variability of the bacilli in their behaviour towards various carbohydrates, and the causes thereof.

We have seen that colonies of this group of bacilli frequently produce daughter-colonies. When this occurs on media containing a carbohydrate, it is often found that the daughter-colonies possess the power of fermenting the carbohydrate, though the mother-culture had shown no capacity to do so. The carbohydrate in the medium is not always the stimulus to the variation, for daughter-colonies on plain agar may show a similar increase of fermenting power. This behaviour, which is similar to that of *B. coli mutabile*, described by Massini in 1907, is largely responsible for the apparent inconstancy of the reactions of the Flexner group in maltose, saccharose, dextrin, raffinose, arabinose, isodulcitol (rhamnose), sorbitol, and probably dulcitol. Daughter-colonies have also been seen on agar containing erythritol, adonitol, and inulin (Twort, 1907 ; Müller, 1911 ; Bernhardt, 1912 ; Winter, 1912 ; Lentz, 1913). An interesting attempt to bring these phenomena into line with the principles of Mendelian inheritance has been made by Stewart (1926, 1928).

A race of bacilli prone to this variation (for it seems that not all strains are) grows into two forms : (1) A ' mixed ' growth, the colonies of which do not themselves change the colour of the carbohydrate-containing medium, but sooner or later produce daughter-buds that do so. Sub-cultures from such colonies reproduce the same phenomenon indefinitely. (2) A pure variant form derived directly from the daughter-colonies. This form ferments the carbohydrate regularly and rapidly, and breeds true unless subjected to violent changes, such as passage through animals (Lentz, 1913 ; Calalb, 1925). The term ' mutation ' is not applicable to this kind of predictable phenomenon, now known to be widespread in the bacterial world. Even ' variation ' must be stripped of all prejudice before it can be safely applied. But, whatever name we choose to give it, the phenomenon obviously forbids the use of single fermentation tests on a wide range of carbohydrates as a means of classification of this group of bacilli. Only those fermentable substances that are acted on by the strain in all its modifications can be used for this purpose.

A variation in the opposite sense to that just described is recorded by Ledingham (1917) under the name of ' reversion '. Actively fermenting colonies of a variable strain were seen to produce non-fermenting (white) daughter-colonies. The observations were only of a preliminary nature, and further work on the subject would be profitable.

Maltose and saccharose have in various countries been considered useful for differentiating the members of the Flexner group. Hiss and Russell's Y bacillus was believed either to fail altogether to ferment *maltose*, or at the most to do so slowly and feebly, whereas ' Flexner ' strains were said to produce acid rapidly and intensely. The bacillus of Strong reacted like ' Y '. According to the same school of thought the ready fermentation of *saccharose* by the Strong type served to distinguish it from Flexner's bacillus, whose action on the sugar was slow, and from ' Y ', which had either no action at all, or an insignificant one. The error of this view is demonstrated by the fermentative irregularity described above, and by

the observation of Hehewerth (1916), Martin and Williams (1917), Barber (1913), Thjøtta (1917), and others, that the initial behaviour of a strain towards saccharose may be reversed after a period of cultivation. Andrewes and Inman (1919) say ' It seems now impossible to decide what this type (i.e. Strong's bacillus) really was '.

Dulcitol, dextrin, &c. Although some workers (Andrewes, 1918) have found that *B. alkalescens* may be distinguished from the Flexner group by its fermentation of dulcitol, others (Dudgeon and others, 1919) consider that this reaction affords a most uncertain criterion, since many Flexner strains are found to acidify dulcitol at some period in their life. According to Thomson and Mackie (1917) this is due to variation (' mutation ') of the bacilli, similar to that seen with other carbohydrates. In general dulcitol cannot be said to be of any great value in the differentiation of the group. The same holds good of dextrin and the rest of the fermentable substances that have been used at one time or another. Nor are the organic salts that have recently been introduced as test-substances for other sections of the typhoid-coli group of any value for the dysentery bacilli.

Serological Reactions.

(See also p. 239.)

Production of Antibodies in Animals.

There is no difficulty in producing antibodies to the Flexner group. Toxicity to the smaller laboratory animals, such as rabbits and guinea-pigs is of so low an order that considerable doses of living or dead cultures are borne without disturbance. For instance, agglutinating sera with titres of one in several thousands are readily obtainable from rabbits with two intravenous injections (0·5 c.cm. and then 1·0 c.cm.) at a week's interval of a diluted broth culture of approximately 400 million per c.cm., killed in the cold with 0·1 per cent. formalin (e.g. a ' standard agglutinable culture '). The animal is bled from the marginal vein of the ear on the sixth and again on the seventh day after the second dose, about 40 c.cm. of blood being taken on each occasion. Equally good results are obtained with suspensions killed by 0·5 per cent. phenol, in intravenous doses of 500 millions followed in 5 days by 1,000 millions. The animal may be bled as early as the tenth day after the first dose. Higher titres may sometimes be obtained with three doses of living agar culture (half a loop, two loops and six loops, at 5 or 6 days' interval (Lentz, 1913). The subcutaneous and intraperitoneal routes of injection give poor results.

The horse and other large animals are said to be relatively more susceptible to intoxication by Flexner cultures (Lentz, 1913). In the manufacture of polyvalent dysentery serum a horse already successfully treated with growing doses of Shiga toxin will tolerate mixed injections of living Shiga and Flexner cultures, which can be gradually increased up to 2 or 3 agar tubes each (Coyne and Auché, 1908 ; Flexner and Amoss, 1915). Shiga (1908) carried the immunization of horses up to very high

doses, such as 40 agar cultures of type I (Shiga) or 75 cultures of other types (Flexner group). Further data are given later, under Serum Therapy.

Agglutination.

The Flexner group differs from Shiga's bacillus in that it consists of a large number of races which give rise, when injected into animals, to agglutinating sera of divergent properties. In a word, the group is serologically diverse (Kruse and others, 1907). To its membership must be admitted any race which springs from a case of clinical dysentery, conforms exactly with the cultural and biochemical specification of the group, and gives a reaction of not less than a quarter of the full titre of one or other type-serum. Furthermore it is unjustifiable rigidly to exclude otherwise orthodox races on the ground of a lack of serological relationship to any of the known types ; for our present knowledge of the composition of the group is admittedly incomplete.

The subject came suddenly into prominence in the war, when bacteriologists working with the troops discovered that a large proportion of the mannitol fermenting dysentery bacilli which they isolated were not agglutinated by the ' Flexner ' or ' Y ' sera then available (Martin and Williams, 1917). A reinvestigation of the whole subject was undertaken by Murray (1918) and Andrewes and Inman (1919). Meanwhile parallel researches on the dysentery of asylums had been independently carried out by Gettings (1919). The results all led to the same conclusions—practically the same as those arrived at long before by Kruse and his co-workers (1907)—that the serological properties of the bacilli are the only reliable criterion for classification, and that there are 4 or 5 chief classes of serologically similar races, with some intermediate strains, and a small residue of strains that defy classification. The experiments of Hiss (1904–5) which seemed to show that serological character is correlated with fermenting power, and on which the old classification was based, must now be taken as disproved. It seems that his error was due to the use of too narrow a range of sera, which by chance gave a serological grouping in agreement with the fermentative classification.

The reason why military bacteriologists failed to identify the bacilli serologically was that they were using only two Flexner group sera (' Flexner ' and ' Y ') instead of the necessary four or five. A bacteriological committee appointed by the War Office in 1918 summed the matter up by recommending the acceptance of Andrewes and Inman's classification of the chief classes under the designations, *B. dysenteriæ* (Flexner) V, W, X, Y and Z. These writers had based on a very extensive series of cross-agglutination and reciprocal absorption tests, performed with 12 sera on 116 strains of bacilli from all over the world, the theory that the antigenic part of every Flexner race is composed, in varying proportions, of four (or five) different antigens, which may be called the V, W, X (Y ?) and Z antigenic components. Thus a V race is one that

is agglutinated to approximately full titre by a V serum, and absorbs all the agglutinins from it. Reciprocally, the serum made with this race gives complete agglutination and absorption reactions with the stock V culture. And so with the other types.

The vast majority of strains can thus be placed in one of five groups, each of which is composed of antigenically more or less homologous races. We say ' more or less homologous ' because the strains in a group are not always fully identical. The antigenic structure of each group overlaps that of the others. For instance, an X bacillus possesses, in addition to its preponderating X-type antigen, a relatively small quantity of V and Z components. That is to say an X serum co-agglutinates V and Z bacilli to a quarter or half its titre. The other type-sera show similar, though usually less extensive, coagglutinations.

Certain strains are of a mixed type, that is to say sera made with them agglutinate strains of two types equally well and can often be completely absorbed by a mixture of the two. Thus, a certain number of races are V, Z in type, others W, X, and doubtless other combinations are possible. Most strains of X and Z are closely related, so that either accurate cross-agglutinations by a standardized method (e.g. Dreyer's method, as used by Andrewes and Inman), or absorption tests, are necessary to distinguish them. Murray (1918) in fact, put them together in a single group. Some X strains, however, are of very restricted agglutinability and react exclusively with the homologous serum, only betraying their relationship to Z, and also to V, by the wide range of agglutinating power of the sera made with them. The Y group, that is, the races that are best agglutinated by a stock Y serum of the old classification, is rather less homogeneous than the other group, and the sera made with these races give a wider range of relatively strong co-agglutination. It could not be finally decided whether they possessed a fifth (Y) antigenic component, or were simply complexes of the other four. But, since there remained after simultaneous absorption with large quantities of V, W, X and Z bacilli an unabsorbed residue of agglutinin in the Y sera, it is reasonable to infer a special Y component, forming a considerably smaller fraction of the total antigen of the Y races than the corresponding primary components do in the remaining types.

In the absorption of a type-serum with a suspension of another type the titre suffered various degrees of reduction, ranging from about 50 per cent. in the case of agglutinatively related strains, e.g. Z or W sera by Y bacilli, down to none at all, as in the case of W serum treated with X or Z bacilli. Compound absorptions of a type-serum with a mixture of bacilli of the other types always left a strong residue of unabsorbed agglutinin.

Murray (1918) considers the Flexner group to be one species, comprising many varieties. He showed the fundamental antigenic similarity of two of the types (V and W) by superimposed agglutinogenic tests similar to those done by Gordon on the meningococci. A rabbit was injected with

a W type bacillus, and then five days later with a V. In response to the W the animal developed a rising curve of W agglutination, and a concomitant low V-curve. The subsequent injection of V sent up the W titre to a great height, just as though a second dose of W had been given. The V curve also rose to a good titre, but not nearly so high as the W. When a similar experiment was done with two bacilli of definitely distinct species, such as Shiga and W, the picture was quite different, for the injection of the second bacillus did not appreciably interfere with the fall of the curve due to the first, and the two curves crossed as the titre to the second bacillus developed. The superimposition of a Sonne-type injection in a rabbit previously dosed with Flexner W also caused a crossing of curves, but there was evidence of some stimulation of the W agglutinin production, suggesting a closer relationship between Sonne and the Flexner group than between the latter and Shiga.

It is not to be assumed that the analysis of the antigens of the Flexner group into five components is, or claims to be a final and exhaustive treatment of the subject. Both in Andrewes and Inman's and in Gettings' (1919) researches a small residue of culturally typical strains had to be left unclassified. One of these is sometimes referred to as Gettings' bacillus. Davison (1922) and several other workers have come across similarly unorthodox races. Furthermore, the theory of components seems actually to conflict with some of the Andrewes and Inman's experimental data. For instance, it was found that the X titre of an X serum was only reduced to 50 per cent. by absorption with a very heavy dose of V, W and Z bacilli mixed together, though all these bacilli possessed an X component, and should theoretically have been able collectively to exhaust the serum. It might be objected that the dose was not large enough, and that really vast quantities of the mixture would have removed all the X agglutinin. But a curve of absorption by different doses, which is given in the paper, shows that a thirty times smaller quantity of the mixture actually absorbed almost as much agglutinin as the full dose, and since the curve was clearly asymptotic, we may judge that absorption would not have been complete with any dose, however large.

Similarly the V titre of a V serum was absolutely untouched by massive absorption with a Z strain, in spite of the fact that the latter is supposed to have a V component. These instances show that the theory, valuable though it be as a working hypothesis, does not provide a complete explanation of the phenomena.

Antigenic variability. We are too much in the dark about the nature and variability of antigens to expect a full understanding of the subject at present. Many bacteria of the typhoid-coli group have been shown to possess a variety of antigens, some of which are constant, others variable, components of the bacillus. In fact, a single race in one phase of growth may be serologically distinct from itself in another phase.

Some unpublished work of Ainley Walker and the writer showed that Flexner strains can be profoundly changed in their antigenic properties

by growth in their own specific antiserum. Park and Collins (1904) had previously found that temporary 'inagglutinability' by stock sera could be induced by this method, and Marshall and Knox (1906) had confirmed this and added the observation that normal horse serum had a similar effect. Walker and Gardner's experiments were made with the idea that a change of type, e.g. of a V into a W, might occur in these conditions, but their expectation was falsified, since the antigenically altered bacilli were as different from the other accepted types as they were from their old selves. The occurrence of a spontaneous serological change in an X race was observed by the writer in 1923 (unpublished). Starting as a strain of very restricted agglutinative and agglutinogenic properties, the bacillus suddenly acquired a much wider antigenic range and enhanced agglutinability, without having been subjected to any special conditions. Its final state was serologically distinct from its original state and from all the other types. To what extent these phenomena are analogous to the antigenic variability of the Salmonella group remains a question for further research. There is present no evidence of alternative 'specific' and 'group' phases, such as are shown by that group, nor do any of the dysentery bacilli possess the heat-labile agglutinable substance (H) of Weil and Felix; all their antigen appears to be in the heat-stable (O) form.

That the serological characters of Flexner races are generally stable under ordinary conditions is proved by experience in laboratories where stock strains of the various types have been maintained for ten or more years without any fundamental antigenic change. An attempt by the writer to induce changes by daily cultivation in broth with and without carbohydrates for upwards of forty days was completely unsuccessful. The practical aspect of agglutinative variations will be discussed, together with some points of technique, under Practical Diagnosis (p. 235).

Comparison of classifications. The Flexner group has been serologically analysed by a number of workers in different countries, with results that agree in principle but differ in detail and completeness. Table III is based on Kalic's (1927) comparison of three separate classifications of the dysentery group, excluding Schmitz's bacillus. With regard to Kruse's types A and D, Andrewes (1923) states that the former corresponds with the English V, and the latter with W. He, therefore, disagrees with Kalic as to the identity of Kruse's D race.

In Sonne's (1915) classification of the Danish mannitol-fermenting strains the Flexner group proper is divided into only two serological types (excluding rough strains), a grouping which tallies with Thjøtta's (1917). In both cases the use of a wide range of sera would probably have necessitated a further subdivision, but at the same time it is possible that the antigenic structure of the Scandinavian mannitol-fermenters is more uniform than that of Andrewes' and Inman's world-wide collection. Some type strains obtained by the writer from the State Bacteriological

TABLE III.

Comparison of serological classifications.

English (Andrewes and Inman)	German (Kruse)	Japanese (Aoki)	Comments
Flexner V	B ? or C ?	—	= old ' Flexner '
,, VZ	A	—	—
,, W	—	—	—
,, WX	—	—	—
,, X	—	II	—
,, Y	D	I	One of the old Hiss strains.
,, Z	H	X	—
Shiga	Shiga-Kruse	VIII	Shiga
—	—	III and V (identical)	—
—	—	IV	—
Dispar	E	—	— Sonne's bacillus (i.e. his group III).
Ambiguus	I, J	—	Schmitz's bacillus

Notes.—Kruse's other types were not obtainable. Aoki's races, other than those mentioned, were not true dysentery bacilli.

Laboratory in Stockholm, where a biochemical classification has been followed hitherto, betrayed antigenic patterns not identical with, but overlapping those of our five types.

The conclusion we may draw from the evidence just presented is that *the Flexner group may be regarded as a single species, culturally and serologically distinct from the bacilli of Shiga, Sonne and Schmitz, and divisible by agglutination tests into a ' spectrum ' of varieties, the primary colours of which are represented by the type strains V to Z.*

The Reactions of Normal Animal and Human Serum with Flexner Bacilli.

Agglutination. The blood-serum of most animals and man has a considerable clumping action on the majority of Flexner races. The figures to be found in the literature vary widely, owing to differences of method and the great variability of the sensitiveness of the suspensions used. Roughly speaking, rabbit sera agglutinate Flexner suspensions of average sensitiveness in 25 to 50-fold dilution. Corresponding figures for human sera are from 5 to 25, but higher titres are quite common, and the fixing of a ' normal ' limit is a matter of considerable difficulty. Bürgi (1907) places the various species of animals in the following order of potency of their normal agglutinating action : ox, horse, goat, sheep, hen, goose, dog, rabbit, man, guinea-pig. There is a great difference in strength

between the species at the top and those at the bottom of the list. This order holds good not only for the Flexner bacilli but for all bacteria of whatever genus, including cocci. It even appears to hold good for the flocculation of mastic suspensions.

The agglutinating power of a normal serum for any given bacillus can be removed by absorption with that bacillus without removing the agglutinins for other bacilli. This behaviour is similar to that of mixed specific agglutinating sera, though the mechanism is not necessarily the same (Burgdorf, 1925).

Bürgi's researches show clearly that normal agglutinins in general cannot be attributed to subinfections. Healthy serum clumps bacilli in virtue of its physico-chemical constitution independently of any entry of bacteria into the body. The intensity of the action varies from species to species, from individual to individual, and from time to time in the same individual. Above all it varies with the agglutinability of the suspension used for testing the serum. The 'normal' titre for Flexner bacilli is said to be increased by various non-specific stimili, such as repeated bleeding, and possibly by fevers. Thjøtta (1917) found the mean Flexner titre of a group of men who had recently had typhoid fever, four times higher than that of a group who had not. But Loewenthal and Bertkau (1919) could not confirm this. Inoculation is said to act in the same way as an attack of fever (Fraenkel, 1915; Schmidt, 1916; Schiemann, 1916), though some writers have found no increase in inoculated persons (Rose, 1916; Glynn and others, 1917). The level of agglutination is higher in old rabbits than in young ones, and in female rabbits higher than in males (Stubington, 1923). The same is true for the human species; there is seldom more than a trace of agglutinin in the serum of new-born babies, and children have less than adults (Loewenthal, 1912). Women show a higher average titre than men (Ritchie, 1916). The assertion has been made that pregnancy raises the level of normal agglutination; but the experimental data are conflicting and the position remains uncertain (Loewenthal and Bertkau, 1919; Acél and Acél-Vechsel, 1924; Koose, 1924). It has been shown that there is no correlation between height of titre and globulin content (Koose, 1924), but beyond this nothing is known of the physics and chemistry of the subject.

Although 'normal' agglutination in general cannot be attributed to infection, there is little doubt that many unusually high Flexner titres in persons giving no history of dysentery are due to subinfection. Ebeling (1913), during a 'Y' epidemic in a German army corps, found very frequent super-normal titres for that bacillus in the blood of enteric cases and practically always in cases of diarrhœa. Ritchie (1916) tested a number of medical students and laboratory workers and found an abnormal proportion of high Flexner titres. One woman, who was working with dysentery bacilli and had suffered from diarrhœa, showed agglutination of nearly one in a thousand, and her fellow worker also gave a high reaction without having had any intestinal disturbance. Again, Loewenthal and

Bertkau (1919) quote the following figures of von Rumpel, obtained in a Flexner epidemic :

Nature of cases	Total No. examined	Flexner-agglutinins present in serum	Flexner bacillus in stools
Patients with dysenteric symptoms ..	40	35	29
Patients with simple diarrhœa.. ..	17	10	6
Healthy men in same area	17	9	2
Men in other areas, including 10 cases of diarrhœa.	56	5	0

Thus we see that the average level of Flexner titres will vary from group to group of human beings according to their present or past exposure to infection. Soldiers who have been through the infections of war show a high average, and so usually do the populations of mental hospitals. Some further consideration will be given to this subject under the heading of Practical Diagnosis, below (p. 235).

Other normal antibodies. Normal horse sera exhibit protective power against Flexner bacilli (Flexner and Amoss, 1915). They also precipitate with filtrates of old cultures. Goat sera show the same phenomenon, but to a lower degree.

Pathogenic Action of the Flexner Group.

Susceptibility of Animals to Natural and Artificial Infection.

The following animals are susceptible to artificial infection, or rather intoxication, with cultures of the Flexner group : dog, goat, ass, horse, monkey, rabbit, guinea-pig, mouse, and (slightly) rabbit. Of these the only ones that contract the disease spontaneously are monkeys and, occasionally, dogs. The dysentery of the monkey tribe probably occurs only in captivity, especially in laboratories. Human beings can be infected by contact with these animals. Of the larger animals mentioned the horse is the most important, since it is used in the preparation of therapeutic serum. Some Flexner strains are almost as toxic for this animal as Shiga's bacillus, but a horse will stand considerable doses of living Flexner subcutaneously after it has been previously prepared with small doses of Shiga toxin in the course of making a polyvalent antiserum. For the production of pure Flexner antiserum cautious immunization with small initial doses of dead or living bacilli is necessary. Baermann and Schüffner (1909) while immunizing goats with Flexner-group bacilli, lost two animals as the result of a single intravenous dose of $\frac{1}{20}$ of a killed agar culture.

The action of the Flexner bacillus on rabbits has been discussed already (p. 224) in comparison with that of the other dysentery bacilli. In general, this beast is difficult to intoxicate with Flexner bacilli by any route of administration ; but very large doses (up to a whole culture), given by any

method of injection, have sometimes been fatal (Lentz, 1913). The symptoms, pathological changes and distribution of bacilli in the body are similar to those caused by Shiga's bacillus. Paralytic phenomena are, however, less common.

To demonstrate the toxic action of the bacilli on guinea-pigs, intraperitoneal injections are the best, but comparatively large doses of most Flexner strains are necessary. Thjøtta (1917) found that from 1 to 3 loopfuls of 24 hours' agar cultures usually killed guinea-pigs in 24 hours. With broth cultures 10 days old, either filtered or unfiltered, 0·5 to 3·0 c.cm. gave a fatal result in nearly half the animals injected. Of the survivors a few became paralysed in the hind quarters about the tenth day. They were killed and examined, without any internal lesions being detected. One guinea-pig developed an acute non-purulent arthritis of both knee joints about five weeks after the injection, but the synovial fluid proved sterile. When acute death occurs in guinea-pigs after intraperitoneal injection, the pathological condition is one of acute peritonitis with septicæmia and toxæmia. Subcutaneous injection is usually ineffective in these animals; oral administration has only very rarely been successful. One of Thjøtta's young guinea-pigs died with bloody diarrhœa five days after feeding with a Flexner strain.

Mice are not very susceptible to this group of bacilli. It seems that some strains can be strongly toxic to them (Amako, 1908), but this is not the general rule. Infection may occasionally be established by the mouth, and result in bloody diarrhœa, with the bacilli in the stools (Thjøtta, 1917). Some strains exercise a moderately toxic action when injected intraperitoneally. Cats, it appears, cannot be infected by the mouth with Flexner group bacilli.

Toxins.

Owing to the fact that the filtrates of Flexner cultures are very harmless compared with those of Shiga's bacillus, the former have been named the Atoxic Dysentery Bacilli. In very old broth cultures of some races of the Flexner group the presence of poisonous substances can be demonstrated by filtration and injection into susceptible animals. Feebly toxic solutions can also be obtained by extracting agar cultures with distilled water or saline solution (Kraus and Doerr, 1908; Kolle, Heller and de Mestral, 1908). The accepted explanation of these facts is that the Flexner bacillus possesses an endotoxin, but does not produce any soluble exotoxin similar to that of Shiga's bacillus (Flexner, 1921). It is true that Pribram (1918) claims to have demonstrated the production of exotoxin by Flexner bacilli, but his proof is not entirely convincing. Prigge (1926) could find no toxin in broth cultures that, in the living state, were virulent for mice, but he obtained a poisonous substance of an endotoxic nature from 24-hours' agar cultures heated in distilled water to 58° C. for 15 minutes. One may conclude, therefore, that the pathogenic action on animals and man is mainly, if not solely, due to the endotoxin produced by

disintegration of the bacilli. The toxicity of Flexner cultures for animals decreases on prolonged cultivation outside the body, but it may be maintained, and sometimes restored, by intraperitoneal passage through rabbits or guinea-pigs.

Hæmolysin. Some Flexner races can cause lysis of erythrocytes (Dudgeon and others, 1919), but the phenomenon is not constant enough to be useful for classification.

Pathogenic Action on Man.

A general comparison of the pathogenicity of the various dysentery bacilli has been given earlier in the chapter (p. 169). There is no fundamental difference between the symptoms and lesions caused by the Flexner group and those seen in Shiga infections (q.v.). The distribution of the bacilli in the lesions is also the same : invariably present in the intestinal lesions and almost invariably in the mesenteric glands, they are only occasionally to be found in the stomach, spleen, liver or bile passages, and still more rarely in the circulating blood or central nervous system.

Subacute or chronic arthritis is a not infrequent complication of Flexner infections. The joint-fluid usually contains a fairly high concentration of specific agglutinins (Worms, Lesbre and Sourdille, 1926; Clifford, 1926). The last-named author, finding an unsuspected Flexner infection in a case of Still's disease, from which several other children appeared to have contracted a mild Y-type dysentery, searched in other cases of ' idiopathic arthritis ' for evidence of Flexner-group infection. In six of these children, some of whom gave no history of dysenteric symptoms, he was able to demonstrate a supernormal agglutination of Flexner V or Y by the blood-serum or joint-fluid. Specific vaccine-treatment was followed by considerable clinical improvement. It is usually impossible to isolate the organism from the synovial exudate in these cases, although Gram-negative (dead) bacilli are sometimes seen with the microscope.

Cystopyelitis may be due to Flexner-infection of the urinary tract, without dysenteric symptoms (Foerster, 1918 ; Hilgers, 1919, who quotes earlier work).

Chronic Flexner dysentery. Ulcerative colitis and the carrier state. Flexner bacilli have sometimes been recovered from the lesions of ulcerative colitis (Dudgeon, 1923), which is regarded by many authorities as an aberrant form of bacillary dysentery (Hurst and others, 1927). But in the great majority of cases, cultivation from the ulcers produces no dysentery bacilli. The stools are invariably negative and the agglutination test seldom gives any indication of the nature of the infection.

Most writers describe chronicity as being less frequent in Flexner than in Shiga dysentery. The carrier state is considered by Fletcher and Jepps (1924) to be a form of mild chronic dysentery, consistent, in the case of Flexner infections, with apparently perfect health. The wall of the large intestine of carriers, if carefully examined, show ' pus pits ' which are usually shallow, but sometimes extend deep down to the serosa. The pus

contains the living bacilli. Similar collections may be found underneath pigmented scars, and are often called 'retention cysts'. The intermittence of the discharge of bacilli is accounted for by the existence of these pockets and cysts.

The proof that bacilli of the Flexner group cause dysentery.

1. The great majority of persons whose excreta contain Flexner group bacilli are suffering, or have recently suffered, from clinical dysentery. The bacilli are selectively situated in the intestinal lesions.

2. The great majority of those who neither have, nor have recently had, dysentery harbour no Flexner bacilli.

3. The blood-serum of persons suffering from dysentery and harbouring Flexner bacilli in their intestines nearly always gives supernormal values in the agglutination and complement-fixation tests at some time during the disease.

4. A number of instances of accidental laboratory infection of human beings with pure cultures of these bacilli are on record, the result of the infection being indistinguishable from bacillary dysentery (Lentz, 1913; Vincent and Muratet, 1917; Murray, 1918; Lippincot, 1925).

Mechanism of the Spread of Infection—Natural Resistance.

There is no need to treat these subjects in detail here, since the facts given in the section on Shiga's bacillus are applicable to the other dysentery bacilli. But a few further data concerning carriers and subinfections with the Flexner group are worthy of mention.

How common are Flexner carriers? There seems to be great variation in their number in different communities at different times. Rosenau (1927) examined the fæces of over four thousand healthy persons in and around Boston without finding a single carrier. One thousand stool-tests of American soldiers returning from France only revealed dysentery bacilli twice (Cruikshank, 1924–5). On the other hand, Ford claimed to have found Flexner group bacilli in the corpses of quite a number of persons not dying from dysentery (Lentz, 1913); and Loewenthal (1912) discovered Y bacilli frequently in the stools both of asylum inmates and of other healthy persons who had had no intestinal troubles, but whose serum had been found to agglutinate the Y bacillus. It is fairly clear that Loewenthal's population was, in comparison with Rosenau's group, highly subinfected. In military Flexner-group epidemics Mayer (1910) and other observers detected a number of healthy carriers who had never shown any signs of infection, though they had been for weeks under careful medical inspection. The number of carriers increased with the spread of the epidemic, until about half as many carriers as cases were discovered. Most of the carriers appeared to carry only for a few weeks, but some were shown to do so for more than a year (Lentz, 1913). One man fell sick of his infection nine days after it had been detected. According to Morgan (1911) Flexner

bacilli are not uncommonly to be found in the stools of individuals in England who give no history of dysentery, such as convalescents from typhoid fever, or suspected typhoid carriers.

The war disseminated the dysentery bacilli widely throughout the world, and Nabarro (1923[2]) warns us to expect dysenteric infections to be less rare than before. Fletcher and Mackinnon (1919) examined nearly 1,000 men convalescent from all kinds of dysentery and found 5 or 6 per cent. to be carriers of Flexner bacilli. About 1 in 5 of these were ' persistent ' carriers, i.e. were not free from the infection three months after its onset. The excretion of the bacilli was, as usual, very intermittent. The blood-sera of carriers usually agglutinated their own races of bacilli up to a dilution of more than 1 in 80.

It is probable that the bacilli cannot persist for long in the healthy human intestine as mere saprophytes. In persistent carriers, as we have already seen, the bacilli exist parasitically in chronic intestinal lesions.

Distribution of the Flexner-types (see also p. 173). The V, W, X and Z races were commonly found on the Western Front in the war, but Y was never identified there, though it was not uncommon in the East, and turned up in Italy. V and W seem to have predominated in Macedonia (Andrewes, 1923). In England, since the war, all types have been found.

Practical Diagnosis of Flexner Infection.

The methods of direct stool examination, cultural isolation and identification of the bacilli are the same as for Shiga's bacillus.

Identification of bacilli by agglutination-tests. Bacilli that give the cultural and biochemical reactions of the Flexner-group (see p. 221) should be tested with a polyvalent agglutinating serum made either by mixing together five type-sera V, W, X, Y and Z, or by injecting a rabbit with a mixed suspension of all the types. With such a serum the diagnosis of ' Flexner-group ', which is adequate for most purposes, can be rapidly established. If it be desired, for epidemiological or other reasons, to determine the serological type of the bacillus, five separate tests with the type-sera will have to be made. Evidence has been obtained in this way of the separate origin of two simultaneous Flexner epidemics in a large town. If in any locality or epidemic races other than those mentioned are found to be ætiologically active, sera made with such races should be included in the diagnostic scheme. The technical method of performing the test is described elsewhere (Vol. IX).

Inagglutinability. A certain number of Flexner strains are inagglutinable when freshly isolated, but recover their agglutinability after variable periods of cultivation—sometimes a few days, sometimes weeks or months. It has been reported by Hamburger and Bauch (1917) and Blumenthal (1920) that Porges' method of heating the bacilli to 100° C. for 30 to 60 minutes may restore the agglutinability of previously inagglutinable dysentery (Shiga or Flexner) bacilli.

Permanent inagglutinability is a proof that the race is serologically distinct from all the type-races used in preparation of the test-sera. We have already seen that this does not absolutely exclude the bacillus from the Flexner group, since the antigenic range of the type-races is probably insufficient, by a small margin, to cover the whole group. But the great majority of such aberrant strains will be found, on thorough examination, to exclude themselves from the group by some divergent cultural or biochemical character.

According to d'Herelle (1926), temporary inagglutinability is a regular result of the action of bacteriophage, whereby a ' resistance-reaction ' is called forth in the bacilli. This causes the formation of a kind of capsule, which prevents the flocculation of the bacilli. It is alleged that permanently inagglutinable variants or ' mutants ' may arise in this way. The power of absorption of these inagglutinable bacilli sometimes remains unimpaired (Flu, 1923), which explains how the absorption test may occasionally clinch the diagnosis when simple agglutination-tests have failed. The ' mucoid ' or capsulated dysenteroid bacillus described by Fletcher (1920) had a peculiar and inexplicable combination of characters : agglutinability without absorbing power. Since it showed an atypical, adherent growth on agar, it is uncertain whether it was a true dysentery bacillus at all. Apart from total inagglutinability, a wide quantitative variation in degree of agglutinability is common in this group ; different colonies from the same stock may vary five- or ten-fold in this respect.

Spontaneous agglutination—Paragglutination. According to Lentz (1913) the microscopic method of agglutination is vitiated, in work on the dysentery bacilli, by the common occurrence of spontaneous flocculation. This difficulty does not arise, in our experience, when formolized broth cultures are used, except in the relatively rare case of the culture being in the ' rough ' state.

The phenomenon of paragglutination, i.e. the agglutination by specific Flexner sera of bacilli of unrelated species or genera, has been recorded a number of times (Duval and Shorer, 1904 ; Ebeling, 1913) (see under *B. coli* and Diagnosis of Enteric Fevers).

The Widal Reaction in Flexner Dysentery.

Acute infection. The detection of acute Flexner infection by the agglutination test of the patient's serum is more difficult than the corresponding serodiagnosis of Shiga dysentery or typhoid fever. The serological complexity of the Flexner group, still not fully unravelled ; the relatively low titres reached by patients' serum, seldom above 1 in 500, and usually considerably less ; the high level of ' normal ' agglutination, even in populations apparently free from dysentery ; and the tendency for coagglutination of Flexner bacilli to occur in Shiga infections—all these factors unite to complicate and confuse the issue. Some authorities, indeed, regard the test as of little assistance in clinical diagnosis. The reaction may be negative in typical cases, even when the bacillus is isolated from

the stools, and it may be positive with the serum of a person who gives no present nor past history of dysentery. But even the Widal reaction for typhoid fever is not altogether free from these defects, and on the other hand a great number of workers have found the test of the greatest value both for the diagnosis of individual cases and for the identification of epidemics.

If the diagnosis by the isolation of bacilli from the stools were easy and certain, no one would trouble with the Widal test; but in reality stool-diagnosis is itself a difficult and delicate procedure, which takes at least 24 hours for a provisional diagnosis, and from three days to a month for a final identification of the bacilli isolated. When all this is done, the failures of this method may be assessed at anything between 20 and 50 per cent., according to the circumstances and the skill and industry of the worker. TenBroek and Norbury (1916) found that stool cultures in the dysentery of children in the U.S.A. failed in 30 per cent. of the cases, but evidence of Flexner infection could be obtained in about two-thirds of the bacteriologically negative cases by means of the Widal reaction with an adequate range of cultures. It would serve no purpose to quote the large number of workers who advocate, and the strong minority who reject, the reaction. The general trend of present day opinion is that it has a very positive value if used with full knowledge of its technical difficulties and diagnostic limitations. Some recent efforts to overcome the more important obstacles may now be described.

Level of diagnostic titre. We have already seen (p. 229) that any person's serum may agglutinate a given Flexner bacillus, and that the height of the titre depends on (1) the agglutinability of the race of bacilli, and of the particular suspension tested; (2) the age, sex, exposure to dysenteric infection, &c., of the individual. And since, owing to these causes, the titres given by different 'normal' individuals with a strain of average agglutinability vary enormously, e.g. from negative at 1 in 2 to positive at 1 in 150, the fixing of a precise line dividing positive from negative reactions is impossible. One person's positive is another's negative. Nevertheless, considerable success has been achieved by the use of Dreyer's method (see Vol. IX), in which (1) the agglutinability of the dead and durable suspensions (Gardner, 1918) is measured against an arbitrary standard representing the average agglutinability of that race; (2) the standard suspension has been tested with an adequate number (about 100) of 'normal' human sera, and a titre-line drawn which will exclude practically all 'normal' reactions. Since the sensitivity-ration of two suspensions of the same race is approximately constant whether specific or 'normal' serum be used in comparing them (Gardner, 1921; confirmed in later experience), there is no need to test with normal sera any suspension except the first of a series, for when the later ones are standardized for sensitiveness, each against the one before, by means of specific agglutinating serum, their ratio of agglutinability by 'normal' sera is thereby also ascertained.

Let us suppose that the limit of normal agglutination for a standard suspension of Flexner 'V' is 1 in 30. Since, in Dreyer's system, 10 standard units has been adopted as the diagnostic limit for all the typhoid-coli group infections, we give this suspension the agglutinability factor of 3·0; for the units in a serum are calculated by dividing the dilution by the factor, i.e. $30 \div 3 = 10$. All future suspensions of this race are subjected to a careful comparative measurement against this suspension, as standard, or against one of the successive suspensions that has been thus standardized; and each is given its correct agglutinability-factor.

Each type race of the Flexner group is dealt with separately in this way (Gardner, 1923), and suspension-factors are fixed at such values as will make a titre of more than 10 units in males (or 20 in females) indicative, if not absolutely diagnostic, of a present or past infection with a member of the group.

The failure of the reaction in certain frank cases of Flexner dysentery may be due, first, to such weak agglutinogenesis in a person of low 'normal' agglutination that his maximum titre due to the infection does not pass the diagnostic limit. In such cases a certain diagnosis is not obtainable by the Widal reaction, though by means of repeated tests, starting early in the illness, it may be possible to detect a significant rise and fall of several hundreds per cent., e.g. a change from negative at 1 in 10 to positive at 1 in 50. Secondly, it may be due to infection by an uncommon Flexner strain that produces little or no agglutinins for the stock types. This difficulty has been to a large degree met by the increase of the stock types to five. In epidemics where serologically aberrant races are isolated, suspensions of them should be added to the diagnostic outfit. Thirdly, failure may be due to reliance on a single test at a stage of the disease when the highest agglutinating power either has not been reached or has passed by. Here again the value of the reaction is vastly increased by repetitions at 3 or 4 days' intervals, and the charting of a curve. This can only be done successfully if variations of agglutinability are excluded by the use of permanent killed suspensions. The peak of the curve may be expected about the same time as in enteric fever, i.e. in the third week after onset of the disease. The rise of agglutinins probably begins about the third day, and may often be detected by the fifth or sixth day. The agglutinins persist for a good many months after recovery, but dwindle to practically nothing in the course of about three years (Thjøtta, 1917). The method of curve-tracing offers the only hope of serological diagnosis in the case of persons whose serum contains residual agglutinins from a past infection or prophylactic inoculation.

Coagglutination. This subject has already been discussed in dealing with the Group (p. 166). As a guide for practice the following rules may be laid down:

1. When a serum gives a strong positive Shiga reaction, the diagnosis of Shiga infection should be made, irrespective of the presence and concentration of Flexner agglutinins.

2. When no significant Shiga agglutination is detected, but one or more Flexner strains shows clumping well above the normal limit for the suspension used, the diagnosis of present or past Flexner-group infection is justified. It is to be noted that Flexner bacilli are sometimes slightly coagglutinated by Sonne sera, but confusion is not likely to arise on this account. The serum in Schmitz infections may agglutinate Flexner bacilli to the same relatively low titres as it agglutinates the homologous organism (Hirschbruch and Thiem, 1918).
3. The type of Flexner infection cannot be determined with certainty from the character of the serum reactions, though there is a certain correlation between the agglutinins in the serum and the antigenic character of the bacillus in the stools (Davison, 1920[2]). It is difficult to say why the correspondence is not closer, but it is possible that dysentery bacilli are capable of changing their antigenic properties during the course of an infection.

Chronic dysentery. Since the isolation of the bacillus in chronic dysentery is practically impossible without proctoscopy, the agglutination test provides the only hopeful routine method of investigating the nature of the infection. Many workers have found it of great value. Robinski (1925) found a fair degree of correlation between the height of the titre which sometimes reached 1 in 1,000, and the severity of the symptoms. Manson-Bahr (1925) says that ' as a rule serological diagnosis is of more value in the chronic stage of the disease than in the acute and rapidly fatal type '.

Carriers give a positive reaction in the great majority of cases (Ebeling, 1913 ; Olitski, 1923 ; Vazquez-Colet, 1925). When searching a community of persons for carriers, those with negative Widal reactions may be provisionally passed over, and only the positive cases further investigated by stool examination. This effects a great saving of time and labour, for without this test the impossible task of repeated stool platings of the whole community would be necessary.

Active and Passive Immunization, Prophylactic and Therapeutic.

Experimental Immunization of Animals.

(See also Group, p. 166, and Production of Antibodies, p. 224.)

Since the smaller laboratory animals are relatively resistant to intoxication by Flexner cultures, it is an easy matter to immunize them against a given race of the group by any of the recognized route of injection. A slight group-protection against other Flexner races may also result, but this cannot be relied on (see also Cross-immunity ; Dysentery-group, p. 173). In guinea-pigs the immunity following a single dose lasts at least two months, and probably much longer (Gay, 1903–4). Very little

has been done with oral immunization in this group, but it is justifiable to assume that the effect would be comparable to that of Shiga's bacillus.

The active immunization of horses against bacilli of the Flexner group is described by Shiga (1908). Animals solidly protected against one or more members of the group may entirely fail to withstand injections of one of the other types.

Active Immunization of Man. Prophylactic and Therapeutic Vaccines.

Prophylactic vaccination solely against bacilli of the Flexner group is sometimes practised in Flexner epidemics when the possibility of Shiga infections can be excluded with reasonable certainty. Thus Lucksch (1908) claims to have brought a ' Y ' epidemic in an asylum to a rapid termination by means of a monovalent ' Y ' vaccine of the ordinary type. The male side of the establishment was dealt with first, and the epidemic ceased in this section of the population, while continuing unabated on the female side. In England, preventive inoculation against the Flexner group has been done for some years at the West Riding Mental Hospital at Wakefield. Dr. J. S. Bolton informs us that the injection of vaccines made from local strains appears to reduce the incidence of the disease very substantially.

It is more usual for circumstances to demand a mixed immunization against Shiga and the Flexner group. But here the difficulty arises that the use of simple vaccines of Shiga's bacillus is precluded by the severity of the disturbances they arouse. The means of overcoming this difficulty are discussed in the section on Shiga's bacillus, and it suffices to say here that Besredka's (1919) oral vaccination appears to be the most promising means of mixed antidysentery prophylaxis. Gauthier (1924), working for the League of Nations, describes what appears to be a very successful immunization of a refugee population in a mixed Shiga-Flexner epidemic by means of polyvalent orally administered vaccine. Similar successes with the same method have been reported by Pascal (1924), Troude (1925) and Maitra and Basu (1926).

The immunization of children against Flexner dysentery has been attempted with apparent success, by Lade (1921) in an original manner, i.e. by the intracutaneous injection of Bœhnke's (1918) 'Dysbakta', a mixture of Shiga and Flexner bacilli, toxins and antitoxins. In a mixed epidemic, 40 children were vaccinated, and showed no further infections, whereas among 13 unvaccinated controls, 5 cases occurred. By the same route of injection, but with a simple heat-killed polyvalent vaccine Bessau (1925) obtained an apparently solid immunity in children from the sixth day after the second dose without any painful reactions or constitutional disturbance. The two doses both consisted of 0·1 c.cm. of a suspension of one slope-agar culture in 50 c.cm. of saline solution. The second was given eight days after the first. Agglutinins were produced with regularity ; infants developed titres of 1 in 20 to 1 in 40 (rarely 1 in 80) ; older children 1 in 50 to 1 in 600. Similar success was claimed by

Weise (1926). But it must be admitted that none of the writers quoted can claim to have scientifically established the efficiency of this type of vaccination.

A form of polyvalent detoxicated vaccine, administered subcutaneously, is recommended by Vincent (1921). The bacilli are killed by ether, and the resulting suspension is said to cause no severe local or constitutional reactions. An almost pure Flexner group epidemic in a restricted population was combated by the injection of over 2,000 persons with this vaccine, which contained 8 races of Shiga's bacillus and 9 of the Flexner group. A single dose of 500 to 750 million was all that could be given in the time. Protection appeared to take 5 to 6 days to develop, and to reach a maximum about the fifteenth day. The statistics given are :

Unvaccinated persons 70·6 cases per 1,000 with 1·6 per 1,000 deaths.
Vaccinated persons 8·1 ,, ,, ,, 0 ,, ,, ,,

It is, of course, exceedingly difficult to assess the value of such figures, but they are at least encouraging. It is, however, open to question whether the etherized vaccine is always quite so harmless as Vincent suggests ; for Gauthier (1924) gave a trial to dysentery vaccines made by Vincent (presumably etherized) and found them all, including even the pure Flexner vaccine, to cause intense local inflammation, very frequent sterile abscesses, and by no means inconsiderable general disturbance.

Vaccine-therapy in Flexner infections has usually been applied only in the chronic form of the disease. Gauthier (1924), however, found that oral administration of small doses of vaccine at frequent intervals exercised a decidedly beneficial effect in the acute stages of dysentery. He used heat-killed broth cultures of Shiga and Flexner (one strain of each) and gave about 7,000 million bacilli daily, divided into three doses. Two or three days of this treatment was sufficient in mild cases. In chronic dysentery, complete recovery after a long course of this treatment was the rule.

Alivisatos (1925) reports that oral administration of polyvalent heat-killed agar suspensions has an effect in acute dysentery comparable to that of the specific antiserum. He noted, further, a non-specific therapeutic action of the vaccine in enteritis of indeterminate nature.

In chronic dysentery Kauntze (1920) and Acton and Knowles (1924), recommend cautious subcutaneous injections of simple vaccines. The former used a mixed Shiga-Flexner-Morgan suspension, killed with 0·4 per cent. phenol, and found that not only chronic dysenteric lesions yielded rapidly to the treatment, but that it also effected an unexpected cure of external ulcers having no connection with dysentery. Acton and Knowles prefer a monovalent vaccine, autogenous or stock, of the same serological type as the infecting organism.

Finally it must be admitted that other workers, such as Manson-Bahr, Perry and Manson (1922) have seen but little benefit come of vaccine

treatment in any form of dysentery. On the whole the evidence is in favour of the value of vaccines, but suggests that their action is largely, if not wholly, non-specific.

Passive Immunity in Man. Serum Therapy of Flexner-Group Infections.

It is the belief of some authorities that serum therapy is of little or no value in Flexner-infections; either because of their relatively low virulence (Dudgeon and others, 1919; Acton and Knowles, 1924), or because the bacilli produce no true toxin and antitoxin treatment is therefore illogical (Kraus and Doerr, 1906).

Fletcher and Jepps (1924) had very poor results with anti-Flexner serum, although administration was early and the serum was polyvalent for all the local Flexner-races. They attribute the failure largely to the debilitated condition of the native Malayans whom they were treating, and consider that the large sum of money expended in serum would have been far better spent in improving the state of nourishment of the infected population. Further accounts of ill success in this sphere are given by Davison (1922). Some of the failures may have been due to the inadequate polyvalency of the serum available, others to delayed treatment.

Results of a far more promising character have been obtained by several workers. Gay (1902–3) and Wollstein (1903) were convinced that Flexner serum had a highly beneficial effect on Flexner dysentery. Ruffer and Willmore (1909, 1910) produced a serum against their El Tor (Flexner-group) bacillus by injecting horses intravenously with an acid-pepsin-digest of mixed strains, which were thereby detoxicated. The results of treatment with this serum were most satisfactory, and with a similarly prepared Shiga and El Tor polyvalent serum they were able to reduce the case-mortality from 64 per cent. to 10 per cent. These authors found that pure Shiga serum had no action on El Tor infections, nor El Tor serum on Shiga.

It is, however, seldom that pure anti-Flexner sera are applicable, for the treatment has almost always to be given without waiting for a precise bacteriological diagnosis. Polyvalent Shiga-Flexner serum is therefore the material of choice. It should be made by means of mixed injections into the same horse, and not by the mixture of separate Shiga and Flexner sera, as recommended by Shiga (1908), since this procedure risks the weakening of the vital Shiga-antitoxin.

A critical review of serum therapy in Flexner-group dysentery by means of polyvalent (Shiga-Flexner-' Y ') serum is given by Dobrashian (1918). From a large number of cases of dysentery passing through Malta from Salonica, 140 were selected as bacteriologically proved to be due to Flexner-group infection, and the results of serum treatment, given reasonably early, were compared with those of the routine administration of ' salines ' at a similar stage. The figures, which are given in Table IV, show that the disease ran a distinctly more favourable course in the cases which received serum than in those treated only in a non-specific manner.

TABLE IV.

Comparison of serum therapy with saline therapy in Flexner-group dysentery (Dobrashian, 1918). Serum-treated cases, 35; saline-treated cases, 67.

	Serum before 10th day	Salines before 10th day
Unsatisfactory convalescence. Per cent. ..	7	22
Relapses. Per cent.	3	14
Carriers. Per cent.	3	6
Cardiac complications. Per cent.	17	15

As to the effect of serum in the chronic stages of Flexner dysentery opinions are divided, but the majority of authorities find it of little value (Manson-Bahr, Perry and Manson, 1922; Webster and Williams, 1925; Dudgeon and others, 1919). Good results have, however, been reported in ulcerative colitis by Hurst (1927) and Ryle (1927). Hurst gives intravenous doses of 40, 60, 80 and 100 c.cm. on successive days, and continues the last dose for a few further days. Rose (1916) found polyvalent serum useful in the treatment of chronic diarrhœa and complications such as arthritis, neuritis, &c., following Shiga or Flexner dysentery. Coyne and Auché (1907) claimed success in the treatment of both early and late Flexner dysentery in infants with polyvalent serum. They gave children under 4 years from 10 c.cm. to 20 c.cm. subcutaneously, according to the severity of the symptoms, and repeated the dose on 2 or 3 successive days in all but the mildest cases. For older children the dose was never less than 20 c.cm. Thursfield (1927) also finds serum helpful in the acute dysentery of children; and Webster and Williams (1925) consider it of great value if, and only if, it is given in the first 48 hours of the illness. For this reason they believe that a rapid diagnosis within 24 hours by the agglutination-test of colonies direct from platings is more valuable, even if less accurate, than slower and completer tests.

The mode of action of Flexner-antiserum is probably anti-endotoxic, that is to say, it acts by preventing local damage to the intestine by the disintegration-products of the bacilli.

Summary. Specific antiserum is a useful remedy in the severer forms of Flexner-group dysentery, particularly if it be administered in the earlier phase of the illness. Except in special cases it is most convenient to use it in the shape of a polyvalent Shiga-Flexner serum. Since it is of great importance to avoid weakening the anti-Shiga properties of the serum, it should be made by mixed injections into the same horse, and not by pooling monovalent sera from separate animals. All the known serological types of the Flexner group should be included in the injections.

The Treatment of Flexner Infections with the Bacteriophage of d'Herelle.

D'Herelle (1921, 1926) believes the bacteriophage to be a practically infallible cure for all forms of bacillary dysentery; but he emphasizes the necessity of a high degree of virulence of the 'phage' for the infecting bacilli. Although d'Herelle is able to bring the evidence of two independent workers (Da Costa-Cruz and Pereira) to his support, several others who have given this method a trial have failed to observe any good effect on Flexner-group infections (Otto and Munter, 1921; Davison, 1922; Munter and Boenheim, 1925; Fletcher and Kanagarayer, 1927). The last-named workers used a bacteriophage obtained from d'Herelle which was virulent for all the stock (V to Z) Flexner strains, and also, though rather less so, for freshly isolated bacilli. It was, however, much more powerful against Shiga's bacillus, and it is striking that in the only Shiga case thus treated the bacilli disappeared with great rapidity. We must admit then that the case for bacteriophage therapy in Flexner-group dysentery is not proved, but the subject undoubtedly deserves further investigation.

Antibacterial Measures.

This subject and others that are omitted in the present section are dealt with under the heading of *B. dysenteriæ* (Shiga) (p. 214).

Bacillus dysenteriæ (Sonne).

By A. D. Gardner.

Synonyms. Duval's bacillus; Pseudo-dysentery bacillus E (Kruse); Baerthlein's bacillus; *B. dispar* (Andrewes); genera *Lancoides* and *Dysenteroides* (Castellani).

Definition and Description.

Definition. A bacillus possessing all the characters of the dysentery group (see p. 161), and distinguishable from the other members by the late production of acid in lactose; by the permanent strong acidification of milk, with or without coagulation; and by serological tests.

Lactose-fermenting bacilli closely resembling the Flexner group were first described by Duval (1904) who thought they were probably true dysentery bacilli; and similar organisms were subsequently encountered by numerous workers when searching for dysentery bacilli in stools. Kruse (1907) included them in his classification of the 'pseudo-dysentery' bacilli as race E, but the rest of the world did not accept them as belonging to the dysentery group at all. Andrewes (1918), who suggested the name '*B. dispar*' for these organisms, was of the opinion that they were not true dysentery bacilli, and thought it probable that there are more species than one.

The first to demonstrate their importance as a true cause of dysentery was Sonne (1915), and in consequence his name is justifiably attached to this species. d'Herelle's (1916) 'new' type of dysentery bacillus was

shown by Thjøtta (1917) to belong to the same category. A general description of the differential characters, the prevalence, distribution and pathological importance of the bacillus will be found in the section on the dysentery group at the beginning of this chapter. We need here only discuss and amplify the statements made in that section.

Morphological, Cultural and Biochemical Characters.

The double tendency of Sonne's bacillus (1) to grow on agar in the form of spreading colonies with irregular margins, and (2) to flocculate spontaneously in broth and in normal saline solution, is probably due to a peculiar composition of the surface layers of the organism. According to White (1927) the second of the two phenomena is caused by a reaction between the serum or the salt and an alcohol-soluble salt-sensitive lipoid substance in the bacilli. By treatment of the bacilli with alcohol, ether or chloroform at 50 to 60° C., this hypersensitiveness is removed. True 'rough' colonies, distinguishable from those just described by their extreme granularity, dryness and opacity, are produced under appropriate conditions. Normal and rough variants are serologically distinct.

Elkeles and Schneider (1927) working with pure line (single cell) cultures describe their 'Kruse E' strains as showing two types of colonies: large rough, and small round. The latter mostly 'sprout' after a time, and produce a secondary growth of the rough type. The round colonies, if plated, always show a mixture of the two kinds of growth.

A production of daughter-colonies may occur on lactose-containing solid media. The primary colonies do not change the colour of the medium, but after some days there appear on them little knobs, which ferment the lactose and take an acid coloration. Subcultures from the knobs give a pure growth of colonies which acidify lactose media rapidly.

It is likely that the delayed lactose-fermentation in fluid media is due to a similar modification of the organism. Ørskov and Larsen (1925) succeeded in splitting a pure-line culture first into two variants (normal and rough), which differed in morphological, serological and biochemical reactions; and then, by subdivision, into four, one of which was almost devoid of agglutinative and antigenic properties. Only the normal variant was clumped by stock Sonne (Group III) serum. In this strain the rough variant seems to have acted like the daughter-colonies described above, i.e. to have shown greater fermenting power than the 'normal' type. Other workers, however, have not found any constant correlation between roughness and increased fermenting power.

Motility. The view of the great majority of workers, including the writer, is that Sonne's bacillus is non-motile. But it should be recorded that Duval (1904) described his bacilli as showing motility immediately after isolation, and that Nabarro has seen it (together with some production of gas) in certain old cultures.

Indole-production. No indole was produced by any of the Sonne strains examined by Thjøtta (1917), Thjøtta and Sundt (1921), Elkeles

and Schneider (1927), Fyfe (1927) and Wiseman (1927). On the other hand, Bamforth (1924) saw traces of indole produced by his cultures, which, although non-pathogenic to rabbits, were otherwise typical Sonne bacilli. The writer's experience favours the negative view; and it is at least possible to say that, for practical purposes, a positive indole reaction provisionally excludes Sonne's bacillus.

Milk and whey. The permanent strong acidification of milk is a constant and reliable function of the bacilli. Subsequent coagulation is variable, depending on the quality of the milk, and perhaps on the fluctuation of the powers of the bacilli. Whey is considered by Sonne (1915) to be a far better and more constant test-fluid than milk. He describes a characteristic reaction of his bacillus (Group III) in whey, which at first turns slightly acid, then returns to neutral, and finally acquires a strong permanent acidity. Thjøtta (1917), however, finds the reactions in whey as variable as those in milk.

Carbohydrates, &c. The following substances, according to Fyfe (1927), are fermented without gas production within 24 hours: glucose, laevulose, mannitol, arabinose, galactose, mannose and maltose. Lactose, in a fluid medium, is attacked either within 24 hours (see above) or at any time up to the end of the fourth week of incubation. On the surface of lactose-containing agar the bacillus may fail altogether to give evidence of acidification, if no daughter-colonies are produced. It has no action on dulcitol, adonitol, inositol, inulin, salicin, sorbitol and xylose. Lead acetate agar is blackened. Saccharose fermentation varies in different colonies; according to Fyfe the round colonies with regular edge act rapidly on lactose and on saccharose, whereas the crenated colonies ferment lactose slowly and saccharose not at all. Since this is the exact opposite of Ørskov and Larsen's (1925) observations, it seems reasonable to conclude that colony-type and fermenting power vary independently.

It is quite likely that some dysentery strains originally classified as 'Strong's' bacillus may have been really of the Sonne type. Hilgers (1920) quotes Kruse as having found that two races of 'Strong' fermented lactose after long cultivation, and Morgan (1911) and Reid (1913) record the clotting of milk by cultures bearing that name.

The close resemblance of Sonne's bacillus to the non-motile form of *B. coli anaerogenes*, and its possible significance have been discussed elsewhere (p. 177).

Serological Characters.

There is not yet sufficient evidence to decide whether Sonne's bacillus is a single entity or a serological group. On the whole the balance is in favour of the former view, which is supported by Kruse (1907), Thjøtta (1917), Hilgers (1920), and Fraser, Kinloch and Smith (1926). In the writer's hands eight strains from four different countries proved to be serologically identical. An illusion of diversity may easily arise if the fact

be ignored that rough and smooth colonies of any organism may be serologically distinct from one another. This and other forms of antigenic variability may perhaps explain the two or more serological divisions described by Sonne (1915), Murakami (1927) and Nabarro (unpublished). For instance, Leuchs and Plochmann (1927) found that only the rough colonies of their Sonne strains reacted with a ' Kruse E ' serum, which may, therefore, be assumed to have been made with a rough strain. A serum made with whole cultures, containing the two modifications, reacted with both. The initial inagglutinability frequently seen in races of this bacillus may also be explained by a too narrow specificity of the serum used.

Agglutinating serums are easy to make in the usual manner unless highly toxic strains are used. Extremely high titres are sometimes obtained, e.g. 1 in 100,000, and are probably due to the hyperagglutinability of the suspensions used in testing (White, 1927). Both by the agglutination and absorption tests the Sonne bacillus is readily distinguishable from the other members of the dysentery group.

Certain Flexner-group serums, particularly the X type, agglutinate Sonne's bacillus to a fraction of their titre, which suggests a genetic relationship.

Pathogenic Action in Animals and Man.

Animals. There is no doubt that Sonne's bacillus is capable of being very toxic to rabbits, guinea-pigs and mice (Thjøtta and Sundt, 1921[1]; Fyfe, 1927 ; Elkeles and Schneider, 1927). Patterson and Williams (1922) describe ulceration of the intestines of rabbits, when death is not too rapid. But there is great variation in the pathogenicity of different races (Hilgers, 1920) which accounts for the negative results of various workers. On the analogy of other bacteria, rough strains are likely to be avirulent. Thjøtta and Sundt (1921[1]), working with autolysates and filtrates of cultures of various ages, believed that they could demonstrate two separate toxins : an exotoxin (the filtrate of a 7 days' broth culture) which produced paresis with regularity in rabbits, and a stronger endotoxin (the filtrate of an autolysed 24-hours' agar culture) causing bloody diarrhœa. A few neutralization experiments with antitoxins made with the two toxins suggested that the antibodies are also distinct.

Man. The following evidence that the bacillus causes human dysentery is given by Sonne (1915).

(1) All his numerous strains, save one, were found in cases of dysentery or dysenteroid diarrhœa.

(2) The bacillus was not found in any of the large number of healthy persons examined.

(3) On several occasions evidence of infectivity was afforded by the isolation of the bacillus from two cases that had been in contact.

(4) The bacilli were often found in large numbers, sometimes even in pure culture in the stools.

(5) The agglutination ('Widal') reaction was positive at a dilution of 1 in 25 in 75 per cent. of the cases, and negative in 99 per cent. of normal persons.

(6) Out of three monkeys (*Macacus rhesus*) repeatedly fed with living cultures, one contracted a severe bloody diarrhœa. It was killed, and was found to have a small cæcal ulcer. The bacilli were isolated in pure culture from swollen lymph follicles and from the mesenteric glands.

To this evidence we are now able to add the occurrence of definite pure epidemics of dysentery in which Sonne's bacillus is clearly the causative agent (Fyfe, 1927); so that there can no longer be any reasonable doubt of its ætiological significance.

As regards epidemiology, we need only emphasize that Scandinavia seems to be the main home of the organism, and that the disturbance it causes is, on the average, mild. When dealing with explosive toxic diarrhœa we should not forget that Sonne's bacillus is now widespread in Great Britain.

Practical Diagnosis.

Complete identification by cultural tests is unfortunately too slow to be of much primary medical value, for the characteristic changes in lactose and milk may take several weeks to appear. Colonies of the dysentery type in platings, if they show the primary characters of the mannitol-fermenting group, should be tested with polyvalent Flexner serum and with a reliable Sonne serum. A reaction only with the latter clinches the diagnosis, which may subsequently be verified by the biochemical reactions.

The Widal reaction. It appears that agglutinins are developed with considerable regularity in Sonne infections (Thjotta, 1917; Fyfe, 1927). The rise starts in the first week of the illness and reaches a maximum in the third week. Only suspensions of known agglutinability both to specific and normal sera should be used, since the variation of Sonne's bacillus in these respects is great.

Immunity and Specific Therapy.

Kruse (1907) found that animals immunized against his type A, B and C are not protected against E (i.e. Sonne). Beyond this there is little known of the immunity-relationships of the organism. It has not been found necessary to apply specific therapy or vaccine prophylaxis to Sonne infections, owing to their generally benign character.

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Flexner group and Sonne's bacillus.

(*N.B.—For references not given here see Dysentery Group references, p.* 182.)

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The Production of Toxin and Antitoxin on the Large Scale.

By R. A. O'Brien.

There still remain unsettled many questions of considerable interest in connexion with the immunology of bacillary dysentery.

To what extent is prophylactic vaccine or serum efficient in combating dysentery caused by (*a*) the Shiga-Kruse bacillus, (*b*) the 'mannitol-fermenting group' ? Is the mode of attack of the first group solely through the toxin produced, and if so, is there more than one toxin ? Is an anti-bacterial serum also necessary ? Does the second group produce true toxins and can a true antitoxin be produced ? In order to simplify the work the Health Committee of the League of Nations decided in 1922 to concentrate effort first on the production and satisfactory titration of antitoxic (Shiga) serum.

B. dysenteriæ (Shiga).

The Production of Antiserum.

Antigen for immunization. Different antigens are used by different workers—culture filtrate untreated or 'toxoided' with formalin, dead bacilli grown on solid medium, dead bacilli together with the broth in

which they have grown, and living bacilli. The tendency at present is to emphasize the importance of toxin and to aim at antitoxic serum. By the injection of any of the antigens mentioned antitoxin can be obtained which will protect against toxic filtrate, dead bacilli and living culture ; in practice, toxic filtrate has some advantages. The question whether the Shiga bacillus secretes one toxin or two (endo- and exo-toxin) or more is still a subject of debate. The tendency is to regard the toxin as a single substance, for apparently antitoxin obtained from horses immunized either with filtrate, or dead or living bacilli grown on solid medium, will neutralize any form of toxin and also protect against the living culture.

Strain. All strains appear to make the same toxin and are neutralized by monovalent serum ; some produce potent toxin more readily than others. For antitoxin production a single strain of high toxigenic power is usually chosen. In the author's laboratory a number of strains in both ' rough ' and ' smooth ' forms have been used to make toxin ; no great difference was found, though the ' smooth ' strains, which are readily kept ' smooth ' and are easier to work with, appear to make somewhat better toxic filtrate and ' dry bacillary body toxin '. For ' toxic filtrate ' the chosen strain is inoculated into a shallow layer of nutrient broth ; in this way is obtained a large air surface which appears to favour the production of good toxin. Toxin is produced in tryptic or peptic digest broth ; ordinary peptone nutrient broth is a good medium. Different workers allow the growth to go on for from 7 to 21 days ; the author finds 14 days convenient. The initial pH of 7·5 changes to from 8·5 to 8·8, and the broth after filtration through a bacteriological candle will usually in a dose of 0·01 c.cm. given intravenously kill mice weighing 20 gm. in three to five days. Unfortunately, though certain filtrates may retain their toxicity for some months, others deteriorate rapidly. By drying or by precipitation a stable product can be produced. By saturation with ammonium sulphate (Doerr, 1908) or sodium sulphate (Petrie, 1927) a precipitate may be obtained which contains most of the toxin. This, when ground and dried, is apparently stable; a dose of 0·05 mgm. given intravenously to a 20 gm. mouse will kill in three to five days and 0·15 mgm. a rabbit of 2,000 gm. Formalin (0·75 per cent) will detoxicate the filtrate in 21 days at 37° C. This toxoid is antigenic. Some serologists use mainly bacillary bodies (Wadsworth, 1927, p. 443), commencing with organisms grown on solid medium and killed by heat, later injecting the living bacilli grown on agar. Though the serum so produced may have a high agglutinin titre there is no clear evidence that it is more potent in protecting animals against the toxin or living culture than serum made with toxic filtrate alone. Because of its stability ' dried bacillary body toxin ' was largely used by the International Committee (League of Nations) in its early work. This is made by growing preferably a ' smooth ' strain of the bacillus for two to three days on ordinary nutrient agar, scraping off the growth in distilled water, heating to the minimum degree necessary to kill, i.e. about 60° C. for 10 minutes, drying and

grinding very finely. Of such a powder, 0·04 mgm. killed 6 mice (intravenously) and 0·01 mgm. killed 9 of 18 mice; 0·05 mgm. killed 2 rabbits and 0·025 mgm. 2 of 3 rabbits of 2,000 gms. weight.

Antitoxin.

From each of 20 normal horses, 0·5 c.cm. of serum was obtained; in no instance did it protect mice against one certain m.l.d. of toxin. None of the sera tested in a dilution of 1 25 agglutinated Shiga bacilli. It is not clear, therefore, whether normal horses possess any basal immunity; the readiness with which they tolerate the initial doses of toxic filtrate or bacillary bodies suggests that some slight degree of basal immunity may exist.

Horses are injected at the beginning of a course of immunization with the selected antigen either daily for three days (Wadsworth, 1927, p. 443) or more usually twice or thrice weekly, until with the gradual increase in dosage quantities of 50 c.cm. to 100 c.cm. are reached, whereupon the injections are given once or twice weekly. Some workers use the intravenous method exclusively, some give the bacillary bodies intravenously and the toxic filtrate subcutaneously; others again give all injections subcutaneously or intramuscularly.

After three to four months a satisfactory titre of antitoxin should be reached and the horse is bled in the usual fashion. It appears to be immaterial whether the blood is allowed to clot naturally or whether clotting is prevented by the addition of 0·1 per cent. of sodium oxalate, the corpuscles removed and the plasma clotted by the addition of calcium chloride. The serum possesses the stability of ordinary antitoxins. The usual Banzhaf-Gibson methods of concentration by salt precipitation may be used.

Testing of antitoxin. Guinea-pigs and rabbits have been used, but mice are now commonly employed. Some workers in the past have tested antiserum against living culture injected intraperitoneally, the serum being injected either before or after the culture. At present the method most employed is that of injecting intravenously into mice a 'test dose' of toxin mixed with the dose of serum under test. This test dose of the toxin originally used contained about 10 mouse m.l.d.; as with other toxins, the 'test dose' of subsequent toxins producing the same lethal effect in mice or rabbits when mixed with one unit quantity of a standard antitoxin, will contain the same number of binding units of toxin-plus-toxoid, but the quantity of free toxin may vary widely. The test dose of some toxins in use by the author has been found to contain 15 to 30 mouse m.l.d. The toxin chosen may be toxic filtrate or ammonium sulphite precipitate made therefrom, or the dead bacilli dried and ground to a fine powder. The toxin and antitoxin are mixed, made up to a volume of 0·5 c.cm. and injected intravenously into mice weighing from 16 to 20 gm. after 45 minutes in the incubator at 37° C. Deaths are recorded up to five or seven days.

The Standards Committee of the League of Nations has adopted as a standard an amount of dried antitoxin kept at the Serum Institute at Copenhagen ; 1 c.cm. of a 1 per cent. solution of this dried powder contains 200 units. An average horse will yield serum containing 600 to 1,200 of these units per c.cm.

Mannitol-Fermenting Group.

The clinical dysentery caused by the mannitol-fermenting group of bacilli is a much less serious disease than that produced by the Shiga bacillus. For this reason the attention of serologists has been mainly concentrated on the Shiga group, and also because attempts to demonstrate specific toxins and antitoxins have on the whole been unsatisfactory, nor does the literature yield many convincing instances of success in the treatment of these dysenteries with serum. Though so-called ' polyvalent ' sera are still made by injecting horses with Shiga antigen and also culture filtrate or dead or living bacilli of the Flexner-Y group, the main effort of late has been spent on standardizing and producing Shiga antitoxin of high potency. There is no generally accepted method of testing for and standardizing curative antibodies to the mannitol-fermenting group of bacilli.

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CHAPTER IV. THE COLON GROUP AND SIMILAR BACTERIA.

By W. J. Wilson (Belfast).

With Sections by W. Bulloch, London Hospital.

The B. coli Group as a Whole.

In this group are included Gram-negative non-sporing, motile or non-motile bacilli which attack carbohydrates with the formation of acid and gas; which fail to liquefy gelatin but frequently form indole and which are found in the intestinal canal in most animals.

Table of Common Characters.

Short rods 0·5 by 1·0 to 2·0μ; frequently motile by means of peritrichous flagella, Gram-negative and non-spore-forming. Hexoses and mannitol are fermented with the production of acid and gas, as are also lactose and frequently saccharose and dulcitol. A classification of members of the group can be effected by a study of the fermentative characters. The growth on agar and gelatin is profuse, moist and spreading and no liquefaction of gelatin occurs. In bouillon and peptone water there is uniform turbidity as a rule and frequently indole is produced. In milk as a rule there is rapid acid formation and coagulation but no peptonization.

List of Members of the Group.

In this chapter an account is given of the following members of the group: *B. coli communis*, *B. lactis aerogenes*, *B. acidi lactici* and certain others which differ in minor characteristics. Though they do not definitely belong to the group, but are closely related to it a description is given of certain paracolon bacilli, *B. cloacæ* and certain non-gas-forming bacteria classified as *B. coli anaerogenes*.

The Relationship of the Group and its Members.

The colon group is a part of a larger typhoid-coli group and the great distinction is that a typical member of the *B. coli* group ferments lactose with the production of acid and gas, whereas *B. typhosus*, *B. paratyphosus* A and B, *B. enteritidis* Gaertner, *B. aertryck*, *B. suipestifer* and the various bacilli of the Shiga-Flexner group of dysentery bacilli are unable to attack this sugar. It will be seen that closely allied to the *B. coli* group are certain strains known as paracolon bacilli which also do not ferment lactose, but in other respects are probably more closely allied to the *B. coli* than to the paratyphoid group. The growth of members of the *B. coli* group and

of members of the group formed by the typhoid, paratyphoid and dysentery bacilli on agar and on gelatin is very similar; all are Gram-negative, do not form spores and morphologically bear a great resemblance to one another.

It is in the first instance by the study of biochemical changes produced in certain media that a classification of the various members of the group and of their relationship to the typhoid-paratyphoid dysentery group can be attained. It is found, for instance, that the typhoid and dysentery bacilli are separated from the others by the fact that they ferment glucose with the production of acid but not of gas. We may take it then that the production of acid and gas is a feature which the colon-paratyphoid group have in common. Starting from this point and following Kligler (1914) the relationship of various members and of closely allied bacteria can be represented as in Table I.

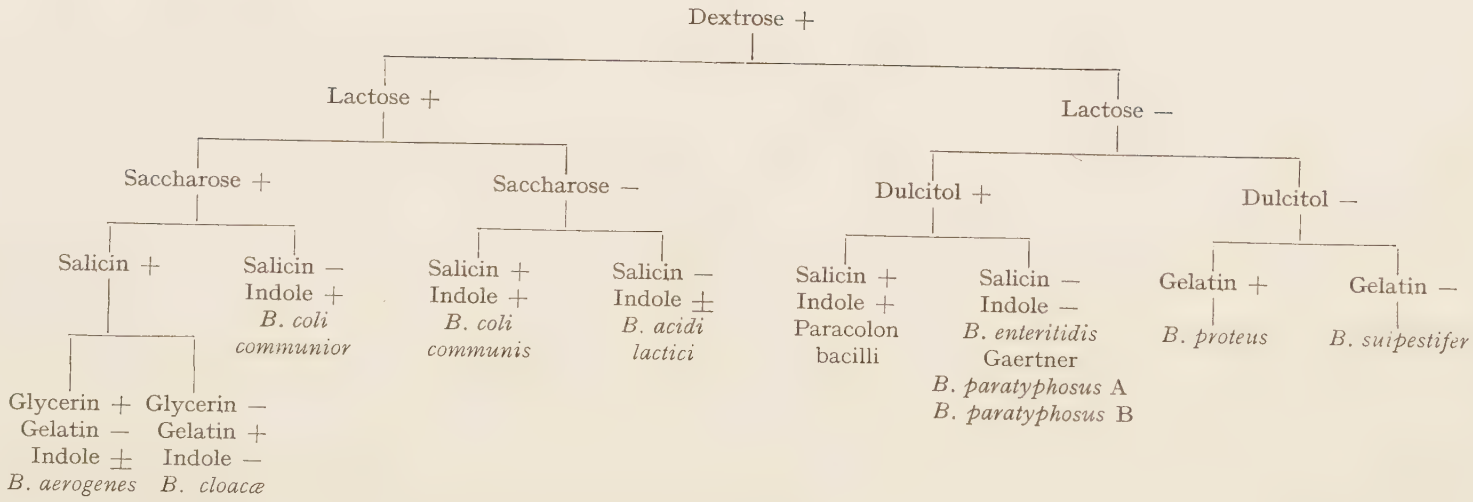
From Table I it is evident that the power to ferment dextrose, lactose, saccharose, salicin, dulcitol and glycerin with the production of acid and gas is useful in classifying bacteria of intestinal origin, and that the power to produce indole and liquefy gelatin is also of value. *B. proteus* and *B. cloacæ* differ from the coli-paratyphoid group, especially in being able to liquefy gelatin. Another important characteristic of the *B. proteus* group is the capacity of its members to split up urea.

The mucoid-encapsulated group is closely related to the *B. coli* group, but is distinguished mainly by the mucoid character of its growth on agar, and by certain sugar and serological reactions. However, it is known that members of the colon and paratyphoid group under certain conditions can be encapsulated and yield a mucoid growth. A connecting link between *B. coli* and the mucoid-encapsulated group is furnished by *B. lactis aerogenes* which is placed by some in one group and by others in the other. Bergey (1926) regards *B. lactis aerogenes* as the type species of a special genus which he designates *Aerobacter* Beijerinck, and considers that *B. coli communis* is a species of the genus *Escherichia* Castellani and Chalmers, and the *B. pneumoniæ* of Friedländer of the genus *Klebsiella* Trevisan.

The *B. alcaligenes* group in its growth on agar is very similar to the organisms of the *B. coli* group, but differs from them in being unable to ferment any carbohydrate.

The two organisms which are most closely related are *B. coli communis* and *B. lactis aerogenes*, and in this chapter *B. lactis aerogenes* will be regarded as forming a subgroup of the *B. coli* group and will not be considered, as it often is, under the mucoid-encapsulated group. Bergey (1926) like many others regards the power possessed by certain micro-organisms and especially by *B. lactis aerogenes* of producing acetyl-methyl-carbinol during the fermentation of glucose as a means of distinguishing them from the great majority of the other members of the *B. coli* group. There is no doubt that by this test deeper and more fundamental differences are shown to exist between the *B. coli communis* and *B. lactis aerogenes* than

TABLE I.


Dextrose +
Lactose +
Lactose —
Saccharose +
Saccharose —
Dulcitol +
Dulcitol —
Salicin +
Salicin —
Indole +
B. coli communior
Salicin +
Indole +
B. coli communis
Salicin —
Indole ±
B. acidi lactici
Salicin +
Indole +
Paracolon bacilli
Salicin —
Indole —
B. enteritidis Gaertner
B. paratyphosus A
B. paratyphosus B
Gelatin +
B. proteus
Gelatin —
B. suipestifer
Glycerin +
Gelatin —
Indole ±
B. aerogenes
Glycerin —
Gelatin +
Indole —
B. cloacæ

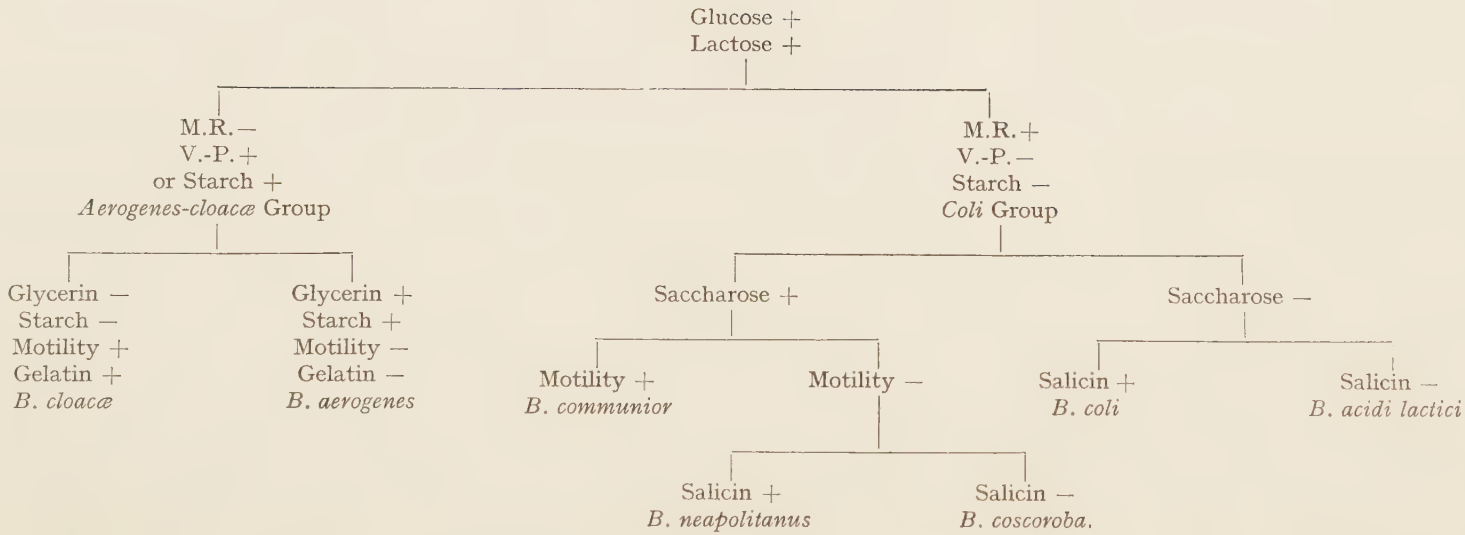
can be shown by merely determining whether a sugar is or is not fermented. In the classification of intestinal micro-organisms the fermentation tests have in practice proved to be sufficiently constant to be of great value. There is something more than chance behind the fact that the majority of the pathogenic races are unable to ferment lactose. When a micro-organism becomes so definitely pathogenic as to be the causative micro-organism in a definite disease, its cultural and perhaps to a lesser degree, its serological characters, become fixed. In the case of the *B. coli* group the members of which are more saprophytic than parasitic there is great variation in their action on sugars and also as will be seen later, serologically. Recognizing that the cultural characters of their growth on agar, potato, bouillon, &c., afford little or no help in the classification of the members of the *B. coli* group, we are forced to make use of what assistance can be afforded by a study of their fermentation and serological reactions and pathogenicity.

Fermentations.

Studies of the actions of members of the *B. coli* group have shown that on carbohydrates and alcohols and glucosides there is the greatest difference in the individual members. If a very large number of these reagents were employed the number of groups found would almost be infinite. Levine (1918) remarked, ' To treat all characters as of equal taxonomic significance leads to an infinite number of unstable varieties ', and, therefore, made use of a coefficient of correlation to determine correlated characters, and in this way succeeded in defining a smaller number of more stable groups. Levine pointed out that as the number of fermentable substances or other characters observed increases the number of varieties increases geometrically—that the number of varieties is given by the formula 2^n where ' n ' is the number of characters studied. Thus, with 8 characters, there are 256 possible combinations, and the number rises to 1,024 with 10 characters, and to 65,536 when 16 characters are observed. It is evident then that certain characters must be found which are of more importance than others, and that a study should be made to ascertain those that are correlated. Before referring to earlier work in this direction, I shall show in Table II the results of Levine's (1918) statistical study of 333 coli-like bacteria isolated from soil, sewage, and the fæces of various animals.

From Table II we see that the characters studied were the fermentation of glucose, lactose, saccharose, salicin, glycerin and starch, the liquefaction of gelatin, the presence or absence of motility and two actions indicated by the initials M.R. and V.P. which are contractions for the methyl red and Voges-Proskauer tests. There are two principal subgroups: (1) the aerogenes-cloacæ group characterized by negative methyl red and positive Voges-Proskauer reactions, or by power to ferment starch: in this subgroup are included *B. cloacæ* and *B. aerogenes;* (2) *B. coli communis, B. coli communior, B. neapolitanus, B. coscoroba* and *B. acidi lactici. B. cloacæ* differs from the other bacteria of the *coli* group in

TABLE II.


Glucose +
Lactose +
M.R. —
V.-P. +
or Starch +
Aerogenes-cloacæ Group
M.R. +
V.-P. —
Starch —
Coli Group
Glycerin —
Starch —
Motility +
Gelatin +
B. cloacæ
Glycerin +
Starch +
Motility —
Gelatin —
B. aerogenes
Saccharose +
Saccharose —
Motility +
B. communior
Motility —
Salicin +
B. coli
Salicin —
B. acidi lactici
Salicin +
B. neapolitanus
Salicin —
B. coscoroba.

being able to liquefy gelatin, but in so many of its characters is it allied to *B. lactis aerogenes* that it is convenient to study them together and to regard them both as a subgroup of the *B. coli* group.

It will now be opportune to consider in greater detail the work that led up to this classification by Levine—a classification on the whole approved of by Winslow, Kligler and Rothberg (1919). Escherich (1885) recognized two distinct types, *B. coli communis* and *B. lactis aerogenes*, the latter being plumper, non-motile and producing more rapid coagulation of milk. Theobald Smith (1893) noted the heavier growth and the tendency to capsule-formation on the part of *B. lactis aerogenes*, and also observed that some forms fermented and some did not ferment saccharose. In a later paper, Theobald Smith (1895) called attention to the ratio of the gases evolved in the fermentation of glucose by *B. coli* and its relatives. This important means of differentiation was confirmed by exact quantitative methods by Harden and Walpole (1906), who, however, obtained somewhat different figures, since Smith had not taken into consideration the loss of CO_2 due to its solution in the medium. Harden and Walpole (1906) found that *B. coli* evolved carbon dioxide and hydrogen in approximately equal volumes, and not as had been observed by Smith in the ratio of 1 to 2. On the other hand, *B. lactis aerogenes* formed twice as much carbon dioxide as hydrogen, and not, as Smith supposed, an equal volume. The difficult gas analyses which this method involved precluded its use for routine purposes until Clark and Lubs (1915) showed that the gas ratio was correlated with hydrogen ion concentration, and that this difference produced by the growth of *B. coli* and *B. lactis aerogenes* in a suitable glucose medium could be easily recognized by the indicator, methyl red. Durham (1901) observed that *B. lactis aerogenes* differed from *B. coli* in being able to ferment starch or inulin and in giving the peculiar reaction described by Voges and Proskauer (1898) in their study of the hæmorrhagic septicæmia group. For the strains of *B. coli* fermenting saccharose Durham proposed the name *B. coli communior.*

Voges-Proskauer Reaction.

Levine (1916) in a paper on the significance of the Voges-Proskauer reaction gives a good summary of what is known of its rationale. Harden and Walpole (1906) found that, besides lactic, succinic and formic acids, ethyl alcohol and carbon dioxide, there is formed from the carbon of the sugar, a crude glycol consisting for the most part of 2 : 3 butyleneglycol (CH_3-CHOH-CHOH-CH_3) which on oxidation yields acetyl-methyl-carbinol (CH_3CHOH-CO-CH_3). The latter is a volatile reducing substance, which when mixed with a solution of potassium hydroxide in the presence of peptone slowly gives rise to an eosin colour. *B. lactis aerogenes* but not *B. coli* is able to oxidize butyleneglycol to acetyl-methyl-carbinol; and to the formation of the latter substance Harden (1905) ascribes the Voges-Proskauer reaction. The eosin-like fluorescence which Voges and Proskauer observed when potassium hydroxide was added

to cultures of certain bacteria in glucose broth is attributed by Harden to a reaction occurring between diacetyl ($CH_3CO.CO\ CH_3$) resulting from the oxidation of the carbinol and some constituent of the peptone.

It is advisable to carry out the test at the end of 1, 3 and 5 days' incubation (Chen and Rettger, 1920) as the acetyl-methyl-carbinol may in course of time be destroyed by the micro-organism in its further growth (Paine, 1927).

Methyl Red Test.

As already mentioned *B. lactis aerogenes* differs from *B. coli communis* in giving the Voges-Proskauer reaction, in other words, in being Voges-Proskauer positive (V.-P.+), and also in producing in glucose broth a ratio of carbon dioxide to hydrogen of 2 to 1 and not 1 : 1, or in other words, belonging to a high ratio group. When cultures are made in this special glucose phosphate peptone solution, and the reaction at the end of five days tested by the use of methyl red, with *B. coli* acid, indicated by a red colour, is produced; in other words, the result is methyl red positive (M.R.+); whilst with *B. lactis aerogenes* the colour is yellow (M.R.−), indicating the production of alkali. Of course, both organisms are able to ferment glucose with the production of acid and gas, so that if the test were employed in the earlier stages of fermentation both would give a methyl red positive result. It would appear that the best results are obtained after 4 or 5 days' incubation at 37° C. The reversion of the reaction in the case of *B. lactis aerogenes* is probably due to the secondary decomposition of organic acids with the formation of basic carbonates and not to neutralization by basic products of protein decomposition (Ayers and Rupp, 1918). The importance of the Voges-Proskauer and methyl red reactions will be shown in connection with the distribution of bacilli of the coli group in soil and water.

The fermentation with acid and gas production of the hexoses, lactose, mannitol, xylose, arabinose and rhamnose, was soon found to be a common property of the *B. coli* group. The use of certain other substances proved of service in the classification of its members. MacConkey (1905 and 1909) employed saccharose and dulcitol for the purpose of subdividing the group into four main types.

Type 1, represented by *B. acidi lactici*, was saccharose negative and dulcitol negative.

Type 2, represented by *B. coli communis*, was saccharose negative and dulcitol positive.

Type 3, represented by *B. coli communior*, was saccharose positive and dulcitol positive.

Type 4, representing *B. lactis aerogenes*, was saccharose positive, and dulcitol negative.

Each type included a number of varieties distinguished by gelatin liquefaction, indole production, the Voges-Proskauer reaction, motility,

fermentation of inulin, adonitol, &c. On very similar lines to MacConkey's classification were those of Bergey and Deehan (1908) and Jackson (1911).

By a study of characters that are correlated it has been possible to reduce the number of subdivisions. Levine's (1918) work is probably the most complete of efforts made for this purpose and its results were utilized in the earlier part of the chapter. The correlation between the fermentation of saccharose and raffinose first observed by Winslow and Walker (1907) has been confirmed by numerous investigators. Kligler (1914) suggested that salicin should be substituted for dulcitol in subdividing *coli*-like bacteria and pointed out that salicin fermentation was more closely correlated than that of dulcitol with the Voges-Proskauer reaction, indole production and gelatin liquefaction. On this basis there are: (1) a saccharose-negative salicin-negative type (generally dulcitol-negative) represented by *B. acidi lactici;* (2) a saccharose-negative salicin-positive type (generally dulcitol-positive) represented by *B. coli communis;* (3) a saccharose-positive salicin-negative type (generally dulcitol-positive) represented by *B. coli communior;* (4) a saccharose-positive salicin-positive (generally dulcitol-negative) represented by *B. lactis aerogenes.*

Kligler considered glycerin of value in separating the *cloacæ* forms from the *aerogenes* bacilli, finding that most of the saccharose-positive salicin-positive glycerine-negative group were gelatin liquefiers, an indication of a reverse correlation between glycerin fermentation and gelatin liquefaction. Stewart (1917) also found a positive correlation between saccharose and salicin fermentation and the V.-P. reaction. *B. lactis aerogenes* sometimes ferments adonitol, but Chen and Rettger (1920) did not find, as Rogers, Clark and Lubs (1918) supposed, in this a means of differentiating fæcal from non-fæcal strains. The power possessed by certain races of *B. lactis aerogenes* to ferment inositol is considered by Mackie (1913) an important criterion in the grouping of coliform organisms.

Abnormal Fermentations; *B. coli anaerogenes*; Paracolon Bacilli; *B. coli mutabile.*

There is no doubt that the fermentation reactions of the *B. coli* group are sufficiently constant to be of service in the classification of its members. No doubt occasionally an organism on prolonged cultivation may lose its capacity of splitting up a carbohydrate formerly attacked by it; but such exceptions are not numerous enough to detract from the value of the method. As a rule the carbohydrate is promptly attacked, and acid and gas are formed in less than 24 hours. Certain strains of colon bacilli were found by Wilson (1910) to require 7 to 21 days at 37° C. to produce an acid reaction in lactose litmus broth, and 2 or 3 days more to produce gas. In the same paper Wilson described a curious form, isolated from the urine, which at 37° C. produced no acid in lactose media and but little gas

in mannitol and maltose ; while at 20° C. it formed an abundance of acid in lactose and an abundance of gas in maltose, mannitol and salicin media, with glucose and saccharose both at 37° C. and at 22° C. only acid was formed. In this case, temperature had great influence on the manner of fermentative activity of the organism. In not developing gas in glucose, but forming some from mannitol, it resembled certain colon bacilli of the '*anaerogenes*' class previously described by Wilson (1908) and regarded by him as connecting links between the *B. coli* and *B. typhosus* groups of organisms. Somewhat similar strains have been found by Patrick (1914) in the urine of typhoid fever patients.

B. coli anaerogenes *Variety.*

Though the production of gas as well as acid is characteristic of *B. coli*, it has been found possible to suppress the production of gas by certain alterations in the environment, e.g., growth in 0·1 per cent. malachite-green broth (Revis, 1911), growth on chloracetic acid agar (Penfold, 1911) exposure to solutions of phenol (0·25 to 0·75 per cent.), NACl (4 per cent.), Na_2 SO_4 (1·5 per cent.) (Smirnow, 1916). Such varieties generally revert to their normal characters on prolonged cultivation on ordinary media, but occasionally the modification remains permanent. Under natural conditions bacilli which in their cultural and fermentative characters resemble *B. coli*, but which ferment the various carbohydrates without the production of gas, have been encountered by numerous observers since Lembke in 1896 isolated such organisms from the intestine of a dog. The papers of Wilson (1908) and Nabarro (1923) contain references to bacilli of this kind described by Cathcart, Dudgeon, Castellani and Mair. Wilson's strains were isolated from the urine in cases of cystitis and pyelitis, and those of Nabarro from stools in dysentery. Some of the strains described by Nabarro were slow lactose fermenters and are believed by him to be identical with Sonne's type of dysentery bacilli.

Paracolon Bacilli.

Although the great majority of the coliform bacilli found in the intestine of man and other animals rapidly ferment lactose with the production of acid and gas, there are strains which in other respects resemble *B. coli* but do not ferment lactose. These form a connecting link between *B. coli* and the paratyphoid group and are commonly designated paracolon bacilli. Trawinski (1924), in a study of 91 strains of the non-lactose-fermenting strains, found that all fermented dextrose, lævulose, galactose, mannose, rhamnose and mannitol, that none fermented saccharose, raffinose, inulin, starch and erythritol, and that according to their action on maltose, xylose, glycerin, dulcitol and sorbitol, milk and litmus whey, they could be placed in eight groups. The members of some of these groups were also identical in their antigenic structure as shown by the agglutination test. All these paracolon bacilli formed indole. Mackie (1913) found that certain non-lactose-fermenters behaved in complement

fixation experiments with an antiserum to a strain of typical *B. coli* in a manner exactly similar to that of typical *B. coli* strains, and suggests that lactose fermentation, which has always been considered the most important criterion of a *B. coli*, can hardly be taken as of any more importance than other sugar reactions. Herrold and Culver (1919) by agglutination tests showed that 43 paracolon bacilli isolated from urine could be placed in four serological groups.

B. coli mutabile.

A curious strain of *B. coli* was studied by Neisser (1906) and Massini (1907), and designated by them *Bacterium coli mutabile ;* it was believed by these observers to show in its relationship to lactose a mutation in the sense of De Vries. When *B. coli mutabile* was grown on solid media containing lactose (Neisser, 1906 ; Massini, 1907 ; Baerthlein, 1912), saccharose (Burri and Düggeli, 1909) or dulcitol, with neutral red as indicator, it formed white colonies with colourless papillæ which turned red if the particular strain varied under the action of the sugar employed. These papillæ are little conical elevations along the surface of the colony and usually require several days' incubation for their appearance. If subcultures are made from the red papillæ on media containing the same sugar, red and white colonies appear. ' The red colonies breed true and never throw white descendants on the same sugar even after prolonged subculture on media not containing it. They may, however, continue to form papillæ from which variations in other characters may arise. The white daughter-colonies continue to form red papillæ which again give white and red colonies in subculture. If a strain which varies, e.g., to lactose, is grown on a medium not containing lactose the papillæ formed do not give descendants varying to lactose, i.e. the stimulus of the homologous sugar is required to produce the corresponding variations ' (Stewart, 1926). In a paper on the Mendelian variation in the paracolon-mutabile-colon group from which the above quotation has been taken, Stewart adduces evidence in favour of the view that the entire series of paracolon, mutabile, colon and pneumobacillus of Friedländer are Mendelian variants of one species. In the paracolon-mutabile-colon series he regards mutabile as the central heterozygote, paracolon as the dominant and colon as the pure recessive and he suggests as a designation for the species *B. neapolitanus* Emmerich, 1885. Such a view would certainly simplify the classification of the almost infinite number of members of the *B. coli* group.

Pathogenic Slow-Lactose-Fermenting B. coli.

That there is some relationship between the power to ferment lactose and virulence is suggested by the fact that the pathogenic paratyphoid group are non-lactose-fermenters and also by Dudgeon's (1924) account of 49 cases of very severe acute infection of the genito-urinary tract in which all the strains of *B. coli* showed delayed fermentation of lactose.

Reduction of Sulphites during Fermentation.

Wilson (1923) finds that unlike *B. typhosus* and *B. paratyphosus* B, most strains of *B. coli* during their fermentation of glucose are unable to reduce a sulphite to a sulphide and thus to produce dark colonies in an agar medium containing glucose, sulphite and an iron salt. A few strains of *B. coli* occurring in every intestine are able to bring about this reduction, and this suggests that the steps in the fermentation process in these strains are different from those of the majority. Wilson and Blair (1927) have introduced a bismuth sulphite glucose iron medium which specially favours the growth of *B. typhosus* and *B. proteus* and inhibits the growth of *B. coli*. The dark colonies formed by *B. typhosus* enable these workers to isolate the typhoid bacillus from stools with the greatest ease.

Serological Reactions.

Agglutination.

It was found by Durham (1897) that the serum of an immunized animal agglutinated the homologous strain to a high titre, but other strains either not at all or in low titre. This has been the experience of numerous other observers, and all attempts to correlate the grouping of strains on a fermentative with that on a serological basis have failed. Strains identical in cultural and fermentative characters usually show themselves distinct as regards the agglutinins they evoke in an inoculated animal. Many investigators have also found that as a rule the agglutination test cannot be employed for the diagnosis of an infection with *B. coli* in the way that so admirably serves with *B. typhosus*.

In recent years the question has again attracted attention, especially since Dudgeon, Wordley and Bawtree showed in 1921 that the hæmolytic strains of *B. coli* met with in infections of the urinary tract were practically all agglutinated by the serum obtained from an animal immunized against a single strain. Their experience with the non-hæmolytic strains was entirely different since these were agglutinated only by the homologous serum. Meyer and Löwenberg (1924) also found that hæmolytic strains of *B. coli* obtained from urinary infections and from the intestines formed a single group serologically, for a few immune sera were sufficient to agglutinate them all. Non-hæmolytic strains were agglutinated by homologous sera only. Indirectly, however, they showed a relationship and common receptor apparatus with the hæmolytic strains in that their sera agglutinated numerous hæmolytic strains. Hees (1926) tested 30 strains of *B. coli* against 3 immune rabbit sera prepared for 2 urinary and 1 fæcal strain of *B. coli*, and found that those which were agglutinated by one serum were agglutinated by the other two sera, but usually in different dilutions. The serum of one of his rabbits agglutinated the homologous strain only in 1 in 40, but a heterologous strain in a 1 in 2,560 dilution. Strunz (1926) found by agglutination and saturation tests that a kinship existed between a number of 23 strains studied by him.

Czickeli (1924) laid stress on the use of a suitable agglutinable strain for the detection of *B. coli* agglutinins. With a certain agglutinable strain he found agglutination occurred with the sera of 16 out of 17 children suffering from pyelonephritis in dilutions from 1 in 40 to 1 in 640, and that this only occurred with 3 out of 100 controls. He also found that the sera of rabbits and rats which had been inoculated with other strains of *B. coli* showed agglutinin for his agglutinable strains. If these findings are confirmed it may be possible to use an agglutinable *B. coli* for the diagnosis of *B. coli* infection of the gall-bladder.

Variation in agglutinability. The great difference between *B. coli* and *B. typhosus* in regard to identification by agglutination would according to Van Loghem (1919) be due to the one being a saprophyte and the other a strict parasite, and according to his view variation in agglutination as in other characters occurs far more readily amongst saprophytes. Anselmi (1924) found support for Van Loghem's view in his study of the agglutinability of single colonies obtained from cultures of two parasitic and one fæcal strain of *B. coli*, as the two strains of *B. coli* which had been isolated from purulent infections remained constant, whilst the colonies of the saprophytic fæcal *B. coli* showed variation in their agglutinability.

Complement Fixation.

Mackie (1913) found that with a *B. grünthal* antiserum with representatives of a non-inositol-fermenting indole-forming group, which included *B. grünthal*, *B. vesiculous*, *B. coli communis* (Escherich), bacilli No. 71 and No. 106 (MacConkey), *B. coscoroba*, *B. neapolitanus*, and two non-lactose-fermenting strains, over 15 doses of complement were deviated in the presence of 0·025 c.cm. of the antiserum ; while with representatives of the other classes in the presence of the same amount of antiserum, not more than five doses were deviated and with some there was no deviation at all. It was also found that these varieties were less resistant than *B. typhosus* to the action of brilliant-green, whilst the inositol fermenters, including *B. lactis aerogenes*, were more resistant.

Pathogenicity as a Means of Distinction.

Inoculation of animals has failed to show any marked difference between members of the *B. coli* group. Strains recently isolated from an infected focus or from an inflamed intestine are usually more pathogenic than strains cultivated from the normal intestine. Dudgeon (1924) describes an acute infection of the urinary tract due to hæmolytic strains of *B. coli* which only slowly fermented lactose. These strains always caused a very acute infection which always cleared up and never became chronic. Bitter and Gundel (1925) concluded that hæmolytic strains of *B. coli* led to a sudden, severe, but short, infection of the urinary tract ; whereas with non-hæmolytic strains the onset is gradual and the disease becomes chronic. This view is not confirmed by Löwenberg (1925).

B. coli communis (Escherichia coli).

History.

By W. Bulloch.

In the course of a bacteriological and chemical research on the fæces of newly-born children, Th. Escherich (1885) found that from the time the children were put on the breast and entirely breast fed, certain bacteria appeared in the fæces. Two of these bacteria were regarded as obligatory components of fæces in that they were always present, and one of them in such numbers that it was almost in pure culture. This was what Escherich called *Bacterium coli commune*. He described it as a short, plump rod, growing easily on gelatin or agar. On potato it formed a slimy mass and it coagulated milk with the production of acid. In an elaborate monograph, Escherich (1886) described *B. coli* in much greater detail and along with it a second obligatory bacterial fæcal element which he named *B. lactis aerogenes*. Escherich drew attention to the general similarity of the two bacteria to *B. typhosus*, and he regarded *B. coli* as a harmless saprophyte. His observations were confirmed early, and subsequent research took two directions: the one, purely technical, dealt with the bacteriological differentiation of *coli*-like bacteria; the other was designed to indicate that *B. coli* might be an active agent in the production of disease. An enormous mass of facts has accumulated with regard to both these lines of work. Within ten years of Escherich's publication, Lösener (1895) gave a bibliography of no fewer than 689 items dealing with the bacteriology of typhoid-coli bacteria. A pathogenic side to the '*coli*' question was first suggested by Laruelle (1889) and has been extensively developed.

W. B.

Salient Characters.

B. coli communis is a Gram-negative, non-spore-forming, bacillus, 0·5 by 1·0 to 2·0μ in size, commonly occurring in the intestine of normal animals, growing readily on ordinary media, fermenting many carbohydrates with the production of acid and gas, and frequently forming indole but unable to liquefy gelatin. When motile it is provided with peritrichous flagella. It is aerobic and facultatively anaerobic, and grows best at 37° C. On agar and gelatin slants the growth is profuse, moist, glistening and bluish by transmitted light. Discrete colonies are opaque, flat or slightly raised with entire or undulate margin. On potato the growth is abundant and greyish to yellowish brown in colour. Broth shows turbidity with heavy sediment but no pellicle, and indole is produced. Acid and gas are formed in dextrose, lævulose, maltose, galactose, arabinose, raffinose, lactose, mannitol, sorbitol, dulcitol, salicin and dextrin.

Differentiation from Allied Bacteria.

By its production of a permanent acid reaction in glucose broth, and by its inability to produce acetyl-methyl-carbinol, *B. coli* is distinguished

from the closely related *B. lactis aerogenes*. From the *B. typhosus*, *B. dysenteriæ* and *alkaligenes* groups it is readily distinguished by the production of acid and gas in various carbohydrate media. From the Salmonella group it is distinguished by ability to ferment lactose and produce indole. The *Proteus* group ferments saccharose and is unable to ferment lactose, liquefies gelatin, and decomposes urea. The mucoid-encapsulated group differs from *B. coli* in being non-motile and in having a characteristic mucoid growth on agar and in not producing indole. *B. lactis aerogenes* is a connecting link between the two groups. We have already referred to the differentiation of *B. coli communis* from other members of the group by means of fermentation of saccharose, salicin, &c. *B. lactis aerogenes* in being methyl-red-negative and Voges-Proskauer-positive is placed by Bergey (1926) in a separate genus—*Aerobacter*—in which is also placed the gelatin-liquefying *B. cloacæ*. These two organisms, *B. lactis aerogenes* and *B. cloacæ*, will be described in a subgroup.

General Position and Importance.

B. coli communis occurs in enormous numbers in the intestines of man and most of the lower animals. No injury to health as a rule results from its presence, but if the bowel wall is damaged by inflammation, traumatism, &c., it may invade the peritoneum and cause acute peritonitis. It is also in certain individuals able to invade the bile and urinary passages, causing cholecystitis, pyelitis and cystitis. Localized abscesses due to the presence of *B. coli* are some times found in the neighbourhood of the anus and genitals. Very rarely is *B. coli communis* responsible for a generalized septicæmia. As a cause of inflammatory conditions of the urinary tract the recognition of the role of *B. coli communis* is most important, as under appropriate treatment the symptoms can be alleviated and the condition frequently completely cured. In pregnancy, pyelitis and cystitis are frequently present and are most commonly associated with the presence of *B. coli*. As an indicator of fæcal pollution of water and milk and shell-fish, much study has been devoted to *B. coli*.

Morphology.

The size of *B. coli* is very variable. In the urine, besides short rods, long threads are frequently seen. In tissues the bacilli are very short and almost coccal in form. In fæces short rods predominate. In young cultures on agar or in broth the usual dimensions of the rods are from 1 to 5μ in length by 0·4 to 0·6μ in breadth. When sugar is present in the media the bacilli are plumper. The addition of certain salts (Péju and Rajat, 1906), dyes (Walker and Murray, 1904), urea (Wilson, 1906) in certain concentrations to the media leads to the development of threads averaging 120μ in length. Wilson described large oval and round swellings occurring in the threads and at the ends of short lateral branches. In these thread forms it is difficult to determine whether true branching is

actually present and not mere close opposition of different filaments. Gardner (1925) has definitely shown that the growth of branching forms of bacilli (' three point multiplication ') is a common occurrence in the stage of rejuvenation of many bacilli, including *B. coli*. It consists of the formation of new, morphologically normal, bacilli, capable of continued growth and multiplication at all three points of a Y-shaped cell. Mellon (1925) regards the swollen forms sometimes seen in cultures of *B. coli* as ' zygospores ' due to the coalescence of the contents of two bacteria and indicative of a primitive form of sexuality. Whether Mellon's observations and his interpretation of them are correct is still undecided.

In the past, most writers were content to label these abnormal shapes as ' involution forms ' and to dismiss them summarily, but it is possible that the life-cycle of bacteria may not be so simple as is generally supposed. Stewart (1928), for instance, suggests that at certain times autogamic conjugation of bacteria occurs and that from the conjugated individuals result spores and daughter-races or papillæ (such as are seen in colonies of *B. coli mutabile*).

Motility and flagella. The typical *B. coli* is usually regarded as motile and described as having four to eight long peritrichous flagella. Young cultures recently isolated from the body may be motile at first and later become non-motile. As a rule the movements of *B. coli* are more sluggish than those of *B. typhosus*. Motility is very frequently absent. Wilson (1908) found that only 6 out of 44 strains cultivated from the urine of cases of cystitis exhibited movement of transposition. Bergstrand (1923) in subcultures on agar from old cultures of *B. coli* observed two types of colonies : one dull and opaque consisting of non-motile bacilli, the other shining, humid and transparent, consisting of motile bacilli.

Capsulated bacilli are non-motile. *B. coli communis* under ordinary conditions is non-encapsulated, but Cooper (1925) claims to have produced capsules not only on *B. coli* but also on *B. typhosus*, *B. fæcalis alcaligenes*, *B. proteus* and *B. cloacæ* by growing them in a medium consisting of glucose broth 9 parts and 1 part of serum or ascitic fluid. He found that motility was lost when capsules were formed. Capsulated *B. coli* were first recognized by T. Smith (1891) and have been developed from ordinary strains of *B. coli* when these were submitted to the action of a bacteriophage (Bordet and Ciuca, 1920 ; Gratia, 1921 ; Gory, 1923). The chemical nature of the capsular substance of pathogenic *B. coli* from bovine sources is the subject of a recent paper by Dorothea E. Smith (1927).

Cultivation.

Temperature. The best and quickest growth of *B. coli* occurs at 37·5° C., when division of the bacilli occurs about every 17 minutes. At 18 to 20° C. growth is slower but well pronounced. Barber (1908) gives as limits at which growth occurs 10 to 49° C. Eijkman (1904) claimed that *B. coli* of human origin was able to ferment glucose broth at 46° C. and employed this temperature in testing water for fæcal pollution.

Reaction. *B. coli* probably grows best at a hydrogen ion concentration of pH 7 but it is tolerant of considerable changes either to the acid or the alkaline side of this point. Dernby (1921) gives pH 6·0 to 7·0 as the optimum range, and the limits between which growth occurs, pH 4·4 to 7·8. Speyer (1924) found that the limits in broth were pH 4·5 to 9·4.

Oxygen requirements. Good growth occurs either in the presence or absence of free oxygen.

Growth in synthetic media. *B. coli* is capable of growing in many simple synthetic media ; for instance, unlike *B. typhosus* it grows in Proskauer-Capaldi medium, No. 1 containing asparagin, mannitol and some simple salts. Koser (1918) found that in a medium containing glycerin, simple salts and uric acid, fæcal strains of *B. coli*, unlike *B. lactis aerogenes*, were unable to attack the purine ring and obtain nitrogen for their growth. Brown (1921) observed that in citrate broth *B. lactis aerogenes* and *B. cloacæ*, unlike *B. coli*, grew more luxuriantly than in plain broth. Koser (1923 and 1924) found that in solutions of simple salts, fæcal strains of *B. coli*, unlike *B. lactis aerogenes*, were unable to obtain from sodium citrate the carbon required for their metabolism. *B. coli* is unable to ferment urea (Paton, 1903). An easy method of differentiating between *B. coli* and *B. proteus* is to grow the organism in bouillon containing 1 per cent. of urea and 0·5 c.cm. of a 0·4 per cent. alcoholic solution of thymol blue for every 100 c.cm. of the medium. In a very few hours the cultures of *B. proteus* become greenish-blue or blue, whilst those of *B. coli* remain yellow.

On *agar* and *gelatin* slants the growth of *B. coli* is moist, slightly raised, profuse and slightly bluish by transmitted light. In shake cultures the deep colonies are round, oval or like a whetstone in shape, and dark in the centre. For a considerable number of years two types of surface colony on gelatin have been recognized : (1) a flat vine-leaf-shaped colony more or less transparent, and (2) a smaller raised round moist colony. Since Arkwright's (1921) work on ' smooth ' and ' rough ' colonies the importance of these types has been recognized. Arkwright's description of the smooth (S) and rough (R) colonies of *B. dysenteriæ*, *B. typhosus*, &c., applies to those of *B. coli* (Gratia, 1922). The S form makes smooth, round, domed, shiny, translucent colonies ; the R form grows in colonies which have a more or less jagged outline, are flatter, often have an irregular rough or dull surface, and are slightly opaque.

Gratia found that variants of *B. coli* produced by action of a lysin showed differences in the character of their colonies and in the antigenic structure of the bacteria, as indicated by agglutination, but there was no alteration in the fermentation of carbohydrates or in the production of indole. Burk (1908) and Baerthlein (1918) had already described variations in the colonies formed by strains of *B. coli* : (1) clear glossy colonies composed of long slender bacilli, and (2) opaque yellowish-white colonies composed of short plump rods. These bacteria showed difference in agglutination.

On *potato* there is an abundant greyish to yellowish-brown growth.

In *milk*, *B. coli* grows rapidly, producing acid and usually clotting, within 3 to 5 days. Certain strains do not cause clotting. The clotting is generally regarded as due to the acid produced, but this may be aided according to Savage and others by a Lab or milk curdling enzyme.

In *bouillon* there is usually produced a uniform and intense turbidity. A pellicle is not as a rule formed but sometimes a film is produced and adheres to the tube at the junction of the fluid with the air. The growth in bouillon of pure cultures of S and R colonies differs. The S form causes uniform turbidity and very slight deposit ; the R form causes a large deposit and sometimes a surface film, but leaves the broth clear.

Action of Selective Substances.

The action of certain concentrations of dyes on the growth of members of the colon-typhoid group has been studied by numerous workers, e.g. Drigalski and Conradi (1902), Loeffler (1906), Conradi (1908), Churchman (1912 and 1913), Browning and Gilmour (1913), Browning, Gilmour and Mackie (1913), Krumwiede and Pratt (1914), Wright (1917), Kligler (1918), and Stearn and Stearn (1924). In general it has been found that *B. coli* is more tolerant of these dyes than *B. typhosus*. An important exception is brilliant-green, which, as shown by Conradi (1908), inhibited the growth of most strains of *B. coli* at a concentration which still allowed the growth of *B. typhosus*. The importance of the hydrogen ion concentration of the medium in determining the selective action was appreciated by Wright, Kligler, and, especially, Stearn and Stearn (1924). Stearn and Stearn state that 'if the bacteriostatic action of basic dyes is of the nature of a loose combination of bacterial matter with difficultly "digestible" dye material we should expect that such bacteriostatic action need not be confined to basic dyes, but that the basic substances of a similar nature should have a similar effect'. They mention that Kligler had found this to be the case in the action of substituted anilines and toluidins, quinoline and quinaldine. Dernby and Davide (1923) found that the bactericidal action of the quinine alkaloids increases with increasing alkalinity, and Kligler (1918) found the same to be true for caffeine. References are given by Stearn and Stearn to numerous observers who had noted that the inhibitory power of basic dyes is increased by increase in alkalinity and decreased by acidity. To quote again from Stearn and Stearn : 'Very striking in this connection are the results of Winslow and Dolloff (1922) on the effect of bile salts on the toxicity of rosolic acid, gentian violet and brilliant-green. These bile salts are sodium compounds of bile acids, as, for example, cholic acid. They should, therefore, be expected to hinder the action of basic dyes and aid, if they affect at all, that of acid dyes. These authors find that the inhibitive power of rosolic acid is unaffected, that the power of gentian violet is reduced from 5 to 50 times, depending on the organism, by the bile salts, and that the power of brilliant-green is reduced by these salts from 200 to 2,000 times'. Wilson and Darling

(1918) had already noted that *B. coli* and *B. typhosus* tolerated a great increase in the concentration of brilliant-green when sodium taurocholate was present in the medium.

It is customary in the isolation of members of the colon typhoid group to make use of basic dyes, e.g. crystal violet, gentian violet, brilliant-green, &c., since these inhibit the growth of many accompanying organisms, especially those that are Gram-positive. Wilson and Blair (1927) have found a sulphite glucose bismuth iron medium which inhibits the growth of many strains of *B. coli*, whilst favouring the growth of *B. typhosus* and *B. proteus*.

Methods of Primary Isolation.

From fæces or infected urine *B. coli* is readily isolated by surface inoculation of lactose litmus agar, Drigalski and Conradi's medium or MacConkey's bile salt lactose neutral red agar. The medium in the neighbourhood of the *B. coli* becomes acid as a result of the fermentation of the lactose. A similar method can be employed for isolation from milk or very impure water.

Lytic Phenomena.

Autolysis. Sturges and Rettger (1922) concluded that *B. coli* undergoes slight changes which may be autolytic in nature, but which at best involves only a small part of the complex nitrogenous constituents of the cells. They mention that Alilaire, Nicolle, and Salimbeni claimed to have observed increase in the soluble nitrogen during the autolysis of *B. coli*, *B. typhosus*, *B. pyocyaneus* and *Proteus vulgaris*.

Bacteriophage. A bacteriophage virulent for *B. coli* is found in the intestinal tract of all animals (D'Herelle, 1918). It has also been found by Dumas (1920) in the drinking water of Paris and by Beckerich and Hauduroy (1922) in the Rhine. Arloing and Sempé (1926) review the literature dealing with the lytic action of certain rivers and oceans on various intestinal bacteria. Bacteriophage is, no doubt, an important factor in the self purification of streams.

Its influence in promoting variation of *B. coli* is referred to elsewhere in this chapter, but here we may mention that D'Herelle (1926) states that under the influence of a bacteriophage filterable forms of *B. coli* —' ultrabacteria '—have been produced by Izar, Tomaselli and Hauduroy. If the reports bearing on the variation of form and function of bacteria induced by bacteriophages are confirmed and established, the whole question of bacterial classification will require profound alteration.

Biochemical Reactions.

Action on Proteins.

B. coli is unable to liquefy gelatin or to digest coagulated blood-serum. It is probably unable to attack the molecules of native albumins or the proteose fractions of Witte's peptone, although it may break down some of the simpler biuret-containing substances in commercial peptone (probably polypeptides) into amino-acids (Sturges and Rettger, 1922).

One of the earliest noted properties of *B. coli* was its power to form *indole* in a medium containing peptone. The presence of sugar inhibits the action, so the medium should consist of sugar-free bouillon or Dunham's simple peptone salt solution. The culture should be incubated for 5 to 7 days. Only about 50 per cent. of strains produce indole. Phenol, creatinine, ammonia, sulphuretted hydrogen, succinic, valerianic, and capric acids may be formed from peptone in addition to indole (Blumenthall, 1895).

Action on Carbohydrates.

B. coli produces acid and gas (composed of equal volumes of CO_2 and H_2) in media containing hexoses, e.g. glucose, lævulose, galactose, mannose, fructose; the pentoses, arabinose, xylose and rhamnose; the disaccharides, lactose and maltose; the polysaccharide, dextrin; the alcohols, mannitol, dulcitol and sorbitol; and the glucosides, salicin and æsculin. Saccharose, raffinose, adonitol, erythritol, and inulin are not attacked. We have already referred to the use of carbohydrates in the classification of the *B. coli* group and have described *B. coli mutabile* and other instances of variation.

Products of fermentation. Reference has already been made to the fact that *B. coli* unlike *B. lactis aerogenes* does not produce acetyl-methyl-carbinol, and, therefore, does not give the Voges-Proskauer reaction, and that equal volumes of carbon dioxide and hydrogen result from the fermentation of glucose. Lactic acid, acetic acid, and alcohol are also formed. According to Harden and Walpole (1906), the formula representing these changes is roughly as follows:

$$2C_6H_{12}O_6 + H_2O = \underset{\text{Acetic acid.}}{CH_3COOH} + \underset{\text{Lactic acid.}}{2CH_3.CHOH.COOH} + C_2H_5OH + 2CO_2 + 2H_2$$

Further details are given in Volume I.

Fermentation of Organic Acids.

As long ago as 1892, Van Ermengem and Van Laer reported that *B. coli* was able to break down formic, succinic, citric and tartaric acids with the formation of hydrogen, carbon dioxide and methane. Koser (1923) in a paper on the utilization of the salts of organic acids by the colon-aerogenes group reviews the literature of the subject and adds many new observations. He found that a number of salts, such as those of acetic, succinic, malic, lactic, mucic, and glycerinic acids, supported an abundant growth of the colon-aerogenes cultures. The most striking result was that in a simple synthetic medium containing sodium citrate, fæcal *B. coli* failed to develop whilst *B. aerogenes* multiplied readily.

Fermentation of Substituted Carbohydrate.

Hees and Tropp (1926) found that many substituted carbohydrates were attacked by all the members of the *coli* group and others by none.

They considered that the use of benzylthioglucoside and glucosethylmercaptal would be of service in differentiation of the members, these compounds being attacked by the *B. lactis aerogenes* but not by *B. coli communis*.

Reduction of Dyes.

By means of reductases *B. coli*, like many other bacteria, can in the course of its growth reduce to colourless leuco-bases many dyes, e.g., litmus, rosolic acid, indigo blue, thionin, methylene blue, toluidin blue, methyl violet, safranin, vesuvin, orcein, neutral red, malachite green, and brilliant-green. The colour is restored on aeration. The dye most commonly employed for diagnostic purposes is neutral red. A glucose agar neutral red shake culture of *B. coli* shows acid and gas and fluorescence in the medium. Cultivation at 46° C. is said to hasten the reaction. Most strains of *B. coli* freshly isolated from excreta show fluorescence, but its presence or absence is not of absolute diagnostic value.

Hæmolysins.

Kayser (1903) found a heat-stable lysin in cultures of *B. coli* and prepared an anti-hæmolysin. Burk (1908) in a study of 139 *B. coli* strains found 8 very actively hæmolytic for goat's blood. Schmidt (1909) using agar plates to which goats' blood had been added, found hæmolytic strains more frequently present in the inflamed than in the healthy intestine. Jaffé (1912) found that 9 out of 97 *coli* strains hæmolysed sheeps' blood agar. A very complete study of *B. coli* infections of the urinary tract especially in relation to hæmolytic organisms was that of Dudgeon, Wordley and Bawtree (1921). They employed a peptone sodium chloride solution, to 5 c.cm. of which 0·1 c.cm. of a thick suspension of washed human blood-cells was added. Of 27 strains of *B. coli* isolated from urinary infections in the male, 20 or 74 per cent. were hæmolytic, while of 42 from females only 11 or 26 per cent. were of this class. All the hæmolytic urinary strains of *B. coli*, with two exceptions, were agglutinated by an antiserum prepared from one strain ; there was no such uniformity manifested by the non-hæmolytic strains. The hæmolysing power was retained with but slight diminution over periods varying from 3 months to 1 year. Klingerstein (1926) also found that the retention of hæmolysin was fairly constant since only 2 out of 10 strains lost this property, and in these it was restored by repeated cultivations on blood agar.

Simchowitz (1926) describes a hæmolytic strain of *B. coli mutabile*.

Serological Reactions.

Antibodies are produced for *B. coli* in the blood of inoculated animals in the same way as for *B. typhosus*. As a rule they are specific for the homologous strain of *B. coli*, and are without action on heterologous strains identical in other characters. Recent work in Germany has confirmed the findings of Dudgeon, Wordley and Bawtree, that the hæmolytic strains of *B. coli* occurring in urinary infections are included in one

serological group as regards the agglutination test. A serum of high titre for one strain of *B. coli* is, therefore, in most instances of no service in determining whether a certain micro-organism is to be regarded as a true *B. coli*.

In the blood-serum of young children there are few agglutinins for *B. coli*, but in people of more advanced age such ' normal agglutinins ' are found. Their presence may be accounted for on the theory that there is a subinfection from colon bacilli harboured in the person's body. The detection of such agglutinins will depend to a considerable extent on the agglutinability of the culture employed. Some strains of *B. coli* are almost inagglutinable and others are very sensitive. Dudgeon, Wordley and Bawtree (1921) using a hæmolytic strain tested the sera of 66 ' normal ' individuals and found 61 negative in a 1 in 50 dilution, 2 were positive in 1 in 50, and 3 were positive in 1 in 400. The urine of 2 of the 5 positive cases was examined and was found to be sterile.

Paragglutination.

It is well known that immunization of an animal against *B. typhosus* often leads to the formation not only of a chief (hauptagglutinin) for the homologous micro-organism but of a partial agglutinin (mitagglutinin) for certain strains of *B. coli*. Saturation with the homologous micro-organism removes such partial agglutinins. Besides partial agglutinins other agglutinins for *B. coli* are found in certain infections. Wilson (1909) in a paper on heterologous agglutinins, more particularly those present in the blood-serum of cerebrospinal fever and typhus cases, reviews the literature and adds instances coming within his own experience. A quotation from this paper reads as follows: ' Paltauf (1904) says the results of Posselt and Sagasser as well as those of Hetsch and Lentz go to show that in the immune serum of animals as well as in that of sick men and women, heterologous agglutinins exist which have no binding groups for the infecting bacteria, but are as specific as regards absorption as those developed in a mixed infection. They must, therefore, be distinguished from partial or mitagglutinins. They can be designated as "heterologous nebenagglutinins", or more briefly as neben-agglutinins. For their formation the view held regarding partial agglutinins does not apply. To explain their origin one must assume that besides the receptors (homologous) that have binding groups fitted to the agglutinogens of the infecting organism, other closely related receptors are set free. In part they would appear to be normal agglutinins whose production through an adequate stimulus is increased '. For the development of heterologous agglutinins there is no satisfactory explanation, but for what are known as paragglutinins there is a theory which has a certain amount of experimental evidence for its support.

Kuhn, Gilderminster and Woithe (1911) cultivated from the stools of dysentery patients *B. coli* strains which were agglutinated to a high titre by an immune serum prepared by inoculation of an animal with a dysentery

bacillus. They termed this reaction paragglutination and believed that *B. coli*, by close association with the dysentery bacillus in the body of the patient had its receptor apparatus modified to resemble that of the pathogenic micro-organism. The modification was usually of a temporary character but occasionally persisted in subcultures for many months or even years (Ditthorn and Neumark, 1913). Instances of paragglutinins of high titre for *B. coli* were found by Park and Williams (1910) and by Kligler (1918) in animals immunized against *B. dysenteriæ* of Flexner and Shiga respectively.

Other Antibodies.

Complement fixation. Reference has already been made to Mackie's work in which he showed that the immune body prepared for a certain strain of *B. coli* was of service in the complement fixation test in the classification of the colon group. As ordinarily carried out the test is not of service in the recognition of strains of *B. coli*, in the way that it is useful for those of *B. typhosus*, *B. pestis*, &c.

Bacteriolysins and precipitins. The marked heterogeneity of the strains of *B. coli* is also seen as regards bacteriolysins and precipitins. Pfeiffer's bacteriolysis occurs when an emulsion of *B. coli* and its homologous serum is injected into the peritoneal cavity of a guinea-pig ; *in vitro* little or no solution of the bacilli occurs (Kraus and Clairmont, 1900).

Pathogenic Action.

In Animals.

The pathogenicity of *B. coli* for guinea-pigs, rabbits, dogs and cats varies with different strains. Strains isolated from the healthy intestine are usually not so virulent as those derived from an inflamed bowel or peritoneum, or from some other inflammatory focus. There would seem to be no great differences in the susceptibilities of different species of animals. In testing virulence, it is usual to employ broth cultures 48 hours old. Of a virulent strain, 0·25 c.cm. injected intraperitoneally or intravenously usually causes the death of a guinea-pig within 48 hours, the symptoms being fall of temperature, collapse, diarrhœa and peritonitis, if the inoculation has been made into the abdominal cavity. Cystitis and pyelonephritis only follow inoculation into the bladder if the urine is artifically suppressed. *B. coli* from the intestine often shows little virulence for guinea-pigs ; thus Houston (1902–3) found only 9 out of 101 strains isolated from normal stools to be virulent. Savage (1906) from his own experiments and from a review of the literature concluded that determining the pathogenicity of *B. coli* isolated from water supplies did not give any additional information either as to the degree of the pollution or as to the dangerousness of the supply as a source of drinking water.

Subcutaneous inoculation of laboratory animals is usually followed by formation of an abscess and no generalized infection.

As ordinary culture of *B. coli* contains bacilli of different degrees of virulence, whether bacilli forming S colonies are more virulent than those composing the R colonies is not definitely settled. In the case of the typhoid-paratyphoid group the S forms are more virulent (Arkwright, 1921 ; Topley and Ayrton, 1924), but Gratia (1922) has found the R form of *B. coli* more virulent. Bordet and Ciuca (1920) have shown that strains of *B. coli* which have acquired resistance to a bacteriophage are less readily phagocytosed and are more virulent to laboratory animals.

Spontaneous infection of animals. Diarrhœa in young calves has been attributed to a *B. coli* septicæmia (C. O. Jensen, 1903) ; Smith and Orcutt (1925) are of a similar opinion and find that the colostrum of the cow affords a certain amount of protection against 'scours' in the calf. *B. coli* may be a factor in the causation of diarrhœa in foals (Gaiger and Dalling, 1921) and in young pigeons (Schneider, 1926).

Analysis of pathogenic action. The usual view held up till recently was that *B. coli* produced no extracellular toxin, and that the toxin was confined to the bodies of the bacteria, and could be liberated by fermentation in the body of the inoculated animal, or outside by autolysis and by repeated freezing and thawing. Vincent (1925), however, showed that at least two toxins are formed—a neurotropic heat-labile exotoxin and a heat-stable enterotropic toxin present in older cultures. Weinberg and Prévot (1927) confirmed Vincent's results and have prepared a serum which is both antitoxic and antibacterial in nature. Steinberg and Ecker (1926) obtained a soluble toxic substance from centrifuged young broth cultures, and prepared an antiserum which afforded a certain amount of protection against the lethal effect of whole cultures.

Pathogenic Action in Man.

Mode of spread and distribution in the body. At a very early stage *B. coli* appears in the intestine of the infant and throughout life man carries without inconvenience millions of these germs in his colon. In health, *B. coli* is confined to the large intestine or at any rate does not extend far beyond the ileocæcal valve into the small intestine. Under normal conditions the mucous membrane of the intestine and its lymph appear to offer a sufficient barrier to the invasion of the body proper. If the bacilli enter the mesenteric circulation they appear to be intercepted by the liver. One of the commonest conditions in which infection with *B. coli* occurs in man is cystitis and in this disease it is said that if blood cultures are made during a rigor *B. coli* is frequently isolated. Cabot and Crabtree (1916), in fact, obtained positive blood cultures in 40 per cent. of their 32 cases under these circumstances. Whether such bacilli gained access to the blood from the intestine or from the bladder lesion is open to doubt. Another diseased condition associated with *B. coli* is cholecystitis. In this case it appears to be almost invariably impossible to cultivate *B. coli* from the blood unless abscesses are present in the internal organs. Posselt (1927) from a review of the literature of the action of

B. coli in cholangitis and cholecystitis concludes that infection passes to the liver directly from the intestine not by the blood-stream. Moynihan (1927) believes that the peritoneal coat of the gall-bladder is first infected presumably from *B. coli* that have passed from the intestine into the peritoneal cavity. The injection of *B. coli* into the healthy bladder or gall-bladder does not cause an infection in animals, but such ensues if the bileduct or urethra are obstructed.

Septicæmia due to *B. coli* is very rare, but it may be found where there is a focus of suppuration, e.g. abscess in the liver, or during a rigor in cystitis, or as an agonal invasion in acute infective processes. In new-born children a fatal condition known as Winckel's disease occurs, and is a hæmorrhagic septicæmia caused by *B. coli*. Felty and Keefer (1924) give an account of 21 cases of septicæmia.

Diarrhœa. Whether *B. coli* or its products can on ingestion cause diarrhœa is still uncertain. In young infants it is very probable that gastro-enteritis may be caused by changes produced in cows' milk by many bacteria, including *B. coli*. The intestine of an individual and the strains of *B. coli* in it appear to become adapted to each other and no symptoms pointing to want of harmony occur. It is possible that foreign strains of *B. coli* when swallowed may cause gastro-intestinal disturbance. The problem of the causation of diarrhœa in infants and as to how far *B. coli* is to be incriminated is far from being settled.

Peritonitis. *B. coli* probably does not pass through the healthy bowel into the peritoneal cavity, but when the bowel is damaged by traumatism or strangulation passage occurs, as of course, also happens in perforation resulting from inflammation and ulceration. In the peritonitis which follows, *B. coli* plays a part along with streptococci and staphylococci. It occasionally is found as the sole infecting agent in abscesses found in the region of the anus and urethra. Its chief importance as a pathogenic agent in man is in connection with inflammation of the biliary and urinary tracts.

Pyelitis and cystitis. The route by which the *B. coli* reaches the pelvis of the kidney and the bladder is still disputed, whether by the urethra or by the blood-stream. Dudgeon, Wordley and Bawtree (1921) discuss the question in the light of their own findings and that of others, and are inclined to believe that in the male infected with a hæmolytic *B. coli*, the route of infection is from the intestine into the blood-stream either direct or by the lymphatics. In the female, probably the infection is more commonly along the urethra. Of later work on the question that of Vincent (1925) points to hæmic infection. *B. coli* may cause a primary pyelitis, and cystitis is often a sequel: occasionally the reverse is the case. In the pelvis of the kidney there is a catarrhal inflammation extending on to the papillæ and in some cases foci of suppuration may occur in the kidney substance. The symptoms of *B. coli* infection of the urinary tract are pain and frequency of micturition and usually a rise of temperature. Not infrequently there are rigors and the patient is acutely,

ill. The urine is strongly acid and often when it is made alkaline by administration of sodium citrate the temperature falls and symptoms of urinary irritation abate.

Cholangitis and cholecystitis. The bile under normal conditions is sterile. Obstruction of the bileducts leads to infection of the biliary tract with *B. coli*. The inflammation may be catarrhal and may be associated with the formation of gall-stones. Posselt (1927) reviews the literature of this subject, which shows that *B. coli* can be but seldom isolated from the blood-stream and so the diagnosis is not facilitated by blood cultures. Occasionally as a sequel to cholecystitis a *B. coli* septicæmia arises, often fatal in its result. The presence of *B. coli* may favour the formation of gall-stones. Moynihan in 50 per cent. of cases of cholelithiasis found *B. coli* present.

Habitation and Viability of the Bacillus.

Distribution.

B. coli is found in the intestine of all warm-blooded animals (except perhaps those of the Arctic regions), and in that of many cold-blooded creatures. Its distribution is, therefore, almost ubiquitous. Coliform bacilli have been isolated from grasses and grains in places where there seemed to be no possibility of fæcal contamination. Probably certain strains of *B. coli* and almost certainly strains of *B. lactis aerogenes* live a saprophytic life outside the bodies of animals. A most important question in connection with the bacteriological analysis of water is to determine whether the strains of *B. coli* isolated are of excretal origin or mere water saprophytes not indicative of contamination. This subject is dealt with in Volume III.

Viability.

B. coli on agar slopes, if protected from desiccation, survives for months or even longer without transplantation, but if a fermentable carbohydrate is in the medium the acidity produced soon destroys the bacilli. Simple drying kills the majority of the individuals in a culture in a few hours or days, but some may survive for 5 or 6 months. Heating to 60° C. kills the bacilli in 10 minutes. If suspended in water and freely exposed to actinic rays of the mercury quartz lamp, death occurs in a few seconds. Sunlight also rapidly destroys the bacilli if conditions are favourable for the penetration of the actinic rays. In soil no multiplication occurs, but weeks or months may elapse before their disappearance (Houston, 1902, 1903).

Skinner and Murray (1926) found that *B. coli* from fresh fæces of cows or from cultures disappeared from soil in from 100 to 200 days.

Very many factors enter into the question of the viability of micro-organisms, e.g. food supply, oxygen, hydrogen ion concentration, salt content, temperature, presence of a bacteriophage, &c. In recent years much work has been done on the viability of suspensions of *B. coli* in

water. Cohen (1922) found a reaction of pH 7·0 most favourable for its maintenance there. Winslow and Falk (1923) observed that there was no material decrease of the organism in distilled water at the end of 24 hours, and that a reaction of pH 6·0 was most favourable for its life, the viability decreasing as the solution became more acid or more alkaline. In their studies on salt action and on the influence of calcium and sodium salts it was found that a sodium chloride solution of 0·0145M strength was favourable to survival. Winslow and Brooke (1927) found that a menstruum containing one part of nutrient broth in 100 parts of water completely abolished the lethal effect of the water, and that this favourable influence was due to the peptone and meat extract ; they suggested that these constituents act as protective colloids. The acidity of carbonated waters and beverages (pH 4·0 to 4·4) explains the fact that after four days no *B. coli* was found in 1 c.cm. though at the start there were 181,000 in this volume at 20° C. (Koser and Skinner, 1922) ; at 1° C. the decrease was slower. Houston (1911) showed that a low temperature favoured the survival of *B. typhosus* in water and Ruediger (1911) noted that colon bacilli were far more abundant in the Red Lake River during the winter when the river was covered with ice than in summer, although the volume of the river and the amount of sewage pollution were about the same.

Natural Resistance of Man and Animals.

The factors concerned in the adaptation that exists between *B. coli* and its host are not at all clear ; even the reason underlying its distribution in the alimentary canal at different levels is not determined. The hydrochloric acid of the gastric juice is usually credited with destroying many of the bacteria ingested in food. It would appear that few *B. coli* are found in the stomach, duodenum and jejunum. In the ileum they increase in number as the cæcum is approached. In the cæcum and ascending colon *B. coli* is found in largest numbers, and beyond the ascending colon the numbers decrease, many of the bacteria found in the fæces being dead. It is thought that individuals become adapted to their own special strains and that foreign strains may occasionally lead to disturbance of health.

Diagnosis and Detection of the Bacillus in Disease.

Cultivation. The isolation of *B. coli* from urine, fæces, pus, &c., can easily be accomplished by planting out on such media as those of Endo, Conradi-Drigalski and MacConkey. It is rarely that *B. coli* can be isolated from the blood, but for this purpose 10 c.cm. of the blood should be added to 100 c.cm. of MacConkey's bile salt broth.

Serological. The difficulties in connection with the agglutinins produced for strains of *B. coli* have already been discussed. In many cases of cystitis there is no appreciable agglutination of the specific strain. Czickeli's (1924) experience with selected agglutinable strains suggests that by the use of such strains a diagnosis might be effected in

cases of cholecystitis and cholangitis or other conditions in which the direct isolation of *B. coli* from the affected organ is impossible. Reference has already been made to the fact that the majority of hæmolytic strains of *B. coli* form a single serological group.

Immunization and Specific Therapy: Treatment with Bacteriophage.

Rabbits can readily be immunized with formol-killed, heat-killed or even live cultures. In the treatment of cystitis and pyelitis the results of vaccine therapy are usually very good. In many cases the bacilli persist in the urine for months but the symptoms of the patient abate. In colitis and sometimes in rheumatoid arthritis (Kauntze, 1925) great benefit is derived from *B. coli* vaccines. Wilson (1913) has found suitable in most cases an initial dose of 20 million or 1/300 mgm. dried bacterial substance. The maximum dose reached in course of treatment does not often exceed 1/40 mgm. Frequently in cases of *B. coli* infection accompanied by fever there is a fall in temperature following the injection.

Antitoxin treatment. Vincent (1925) and Steinberg and Ecker (1926) and Weinberg and Prévot (1927) have prepared antisera against the soluble toxic substance of young cultures of *B. coli*. This serum protected rabbits against five fatal doses of the toxin.

Bacteriophage therapy of colon bacillus infections. D'Herelle (1926) reviews the literature bearing on this subject and gives an account of his own experience. The preparation of the bacteriophage which he used was prepared by the complete bacteriophagy of a suspension of *B. coli* containing 250 million bacilli per c.cm., and of this he gave two subcutaneous injections of a maximum of 2 c.cm. at an interval of 24 or 48 hours. He also instilled a 1 in 10 dilution of the suspension into the bladder in certain cases of cystitis. The results were excellent where the infecting strain was susceptible to the phage and often all symptoms had disappeared and the urine had become sterile at the end of week. Much more experience will, however, be required before the value of bacteriophage therapy can be appraised. An answer is also needed to the question whether the therapeutic results are due to the action of the phage or to the body's response to the antigen of the lysed bacteria.

Other Members of the B. coli Group.

Fermentation tests, as we have seen, are of great service in differentiating the members of the typhoid-paratyphoid-colon groups, but if applied to the *B. coli* group and sufficient sugars, &c., employed the result would be an almost infinite number of varieties. It is of interest to mention a few of these varieties and their chief characters. This can perhaps be most effectively presented in tabular form as was done by MacConkey (1906).

TABLE III.

		Saccharose	Dulcitol	Adonitol	Inulin	V.-P.	Indole	Gelatin	Motility
Group 1	*B. grünthal*	−	−	−	−	−	+	−	+
	B. levans	−	−	−	+	+	−	+	+
	B. acidi lactici	−	−	+	−	−	+	−	−
Group 2	*B. coli communis*	−	+	−	−	−	+	−	+
	B. cavicida	−	+	−	−	−	+	−	+
Group 3	*B. neapolitanus*	+	+	−	−		+	−	−
	B. rhinoscleromatis	+	+	+	−	−	+	−	−
	B. pneumoniæ (Friedländer)	+	+	+	−	−	+	−	−
	B. oxytocus perniciosus	+	+	+	+	+		−	−
Group 4	*B. lactis aerogenes*	+	−	+	−	+	+	−	−
	B. cloacæ	+	−	−	−	+	−	+	+
	B. coscoroba	+	−	−	−	−	+	−	−

+ indicates acid and gas or a positive reaction.

All the organisms in Table III fermented glucose, lævulose, mannose, galactose, lactose, raffinose, mannitol, sorbitol and dextrin; none of them fermented erythritol.

VARIATION.

We have already seen that hæmolytic *B. coli* may show the greatest differences in their fermentative characters and yet their antigenic constitution as shown by the agglutination test may be the same. The dissociation of cultures into S and R strains has already been considered. Under the influence of a bacteriophage and other agencies such dissociation may be readily brought about. By the use of chloracetic acid (Penfold, 1911) and by combined growth with *B. paratyphosus* B (Smith and Smith, 1920) gas production by *B. coli* has been suppressed. No doubt in many cases the variant strain assumes its normal characters as soon as the incitant agent has been removed, but permanent variations have been produced by the action of malachite green (Löeffler, 1906; Lommel, 1926), and of malachite green and brilliant-green (Revis, 1912, 1913). Dawson (1919) found that the addition of a butter soap to the medium led to the loss of power to ferment saccharose in a *B. coli communior* strain, whilst Lommel (1926) found that by the action of phenol a *B. coli communis* strain acquired this power. Esther Stearn (1923) claims to have changed a *B. coli communis* into a *B. coli communior* by the action of gentian violet, and by associated growth with *B. paratyphosus* B and *B. dysenteriæ*.

In addition to its other effects, a bacteriophage may according to Fejgin (1923) and D'Herelle (1926) profoundly alter the fermentative characteristics of intestinal bacilli. D'Herelle describes Fejgin's experience with resistant Shiga strains, where the ordinary bacillus of Shiga mutated into : (1) an inagglutinable Shiga bacillus ; (2) a Flexner bacillus ; (3) a colon bacillus fermenting with gas formation lactose, glucose, maltose, arabinose, lævulose, mannitol and dulcitol, and agglutinable to a titre of 1 : 1,600 by an anti-Shiga serum and by its homologous serum. Before such observations can be accepted as accurate, further and independent evidence must be obtained.

Sonnenschein (1926 and 1927) also regards Friedländer's bacillus, *B. ozænæ* and *B. rhinoscleromatis*, as only mucoid forms of *B. coli*, *B. proteus* 'O' form, *B. paratyphosus* B and *B. dysenteriæ*.

It would seem that variation occurs more frequently than most writers suspected amongst organisms of the *B. coli* group. Arkwright (1913) found a *B. acidi lactici* bacillus changing into a non-gas-producing bacillus in the bladder of a patient suffering from cystitis, and mentions a similar case reported by Sørensen (1912). Such non-gas-producing coliform organisms have been found by Mair (1906) and Wilson (1908) in the urine in cases of cystitis and may in Arkwright's opinion be variants of *B. coli*. Hiss (1904) found that *B. dysenteriæ* Y acquired the power to ferment maltose by prolonged growth in a medium containing this sugar, and Twort (1907) produced similar changes in *B. typhosus* as regards dulcitol and lactose, and in a similar fashion Klieneberger (1927) has 'trained' a coliform bacillus to ferment salicin and æsculin.

Considerations of this nature show the inadvisability of describing as permanent species the large number of varieties of the *B. coli* group, so that we shall content ourselves with giving the salient characters of a few of them.

B. neapolitanus.

This is a non-motile bacillus which differs from *B. coli communis* in fermenting saccharose and raffinose and in not usually fermenting dulcitol.

B. coli communior.

This is a sluggishly motile bacillus with the usual characters of *B. coli communis*, but that it ferments saccharose. *B. coscoroba* may be regarded as a non-motile variety.

B. acidi lactici.

This is a non-motile coliform bacillus which usually ferments adonitol and fails to ferment saccharose, raffinose and salicin. *B. grünthal* may be regarded as a motile variety.

All the above varieties are abundant in human fæces, belong to the low gas ratio type, are methyl-red-positive and Voges-Proskauer-negative, and all produce indole. Robinson (1920) from an analysis of the descriptions given by various observers of coliform lactose-fermenting bacilli

isolated from normal human stools, found that of 2,100 strains the percentage distributions in groups was :

Indole + V.-P. −	Indole + V.-P. +	Indole − V.-P. +	Indole − V.-P. −
91·8 per cent.	1·4 per cent.	4·7 per cent.	1·6 per cent.

The Aerogenes-Cloacæ Subgroup.

The *B. lactis aerogenes* and *B. cloacæ* in the fermentation of glucose produce twice as much CO_2 as H_2 and are methyl-red-negative and Voges-Proskauer-positive. To supplement the descriptions already given the following summary is given of their chief characteristics.

B. lactis aerogenes. A plump non-motile frequently capsulated bacillus forming moist convex viscid colonies, which ferments the hexoses, maltose, xylose, arabinose, rhamnose, lactose, saccharose, raffinose, salicin, usually glycerin, starch and inulin, and sometimes inositol, adonitol, dulcitol and mannitol. It may or may not form indole and does not liquefy gelatin. The exact chemical nature of the capsule of *B. lactis aerogenes* has not yet been determined, but Schardinger (1902) found that it was a gum-like substance containing galactose and glucose. The organism is found in small numbers in fæces and is frequently present in soil and on grains.

B. cloacæ Jordan. This organism in being able to liquefy gelatin differs from the other members of the *B. coli* group and may be regarded as intermediate between the *colon* and *proteus* groups. It is a sluggishly motile non-spore-forming non-encapsulated bacillus, fermenting the hexoses, maltose, mannitol, xylose, arabinose, lactose, rhamnose, saccharose, raffinose and salicin, often inositol but generally not dulcitol, glycerin, starch, inulin or adonitol. From glucose it produces at least twice as much CO_2 as H_2, often the ratio is 5 to 1. In litmus milk there is slow acid formation with coagulation and slow peptonization. It may or may not form indole. It is found in sewage and in human and in animal fæces. Jeney (1927) has described a bacteriophage which acts on the organism.

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The Mucoid-encapsulated Group.

The Group as a whole.

Definition.

In this group are included Gram-negative, non-sporing, non-motile organisms, which ferment a number of carbohydrates, usually with gas production, and which are characterized by the possession of a capsule and a mucoid growth. They are chiefly found in the respiratory tract.

List of Common Characters.

Form ; short plump rods with rounded ends. *Average size ;* rods, 0·5 to 1·0 by 0·6 to 5μ, but may be very pleomorphic. *Motility, flagella, spores ;* absent. *Capsules and staining ;* encapsulated and Gram-negative. *Gelatin ;* no liquefaction. *Litmus milk ;* acid with or without coagulation, or no change. *Agar ;* smooth, moist, sticky, tenacious growth. *Broth ;* turbid, often with a pellicle. *Indole ;* usually not produced. *Optimum temperature ;* usually 37° C.

List of Members of Group.

B. mucosus capsulatus (syn. *B. pneumoniæ ;* Friedländer's bacillus ; *Klebsiella pneumoniæ* Trevisan). *Bacillus of rhinoscleroma* (syn. *Klebsiella*

rhinoscleromatis). *B. mucosus ozænæ* (*Klebsiella ozænæ* (Abel) Trevisan). *Bacterium cyprinicida* Plehn (syn. *Klebsiella ciprinicida* (Plehn) Bergey *et al.*).

Relationships of the Group.

In their growth on various media and in their failure to liquefy gelatin and in their being Gram-negative, as well as in their action on various carbohydrates the members of this group show a kinship to that of the *B. coli*, and particularly to the *aerogenes* type. Coulter (1917) compared the cultural characteristics of this group with those of the *aerogenes* type of *B. coli* by growing the organisms on the surface of dextrose agar contained in large plates. Colonies of the Friedländer type were gray, translucent and of a syrupy consistence, whilst those of the *aerogenes* type were ivory white and of a more pasty consistence.

The possession of a capsule and of an intracellular mucoid matrix is the group characteristic, but it is noteworthy that under certain conditions, *B. coli* and B. *paratyphosus* B may develop in a similar fashion. Thus Revis (1910) observed that the prolonged growth of *B. coli* in soil contaminated with human fæces and sterilized, led to the appearance on plating of large mucoid colonies. Subjected to the action of a bacteriophage, it may also undergo a mucoid transformation (Bordet and Ciuca, 1920 ; Gratia, 1922 ; D'Herelle, 1926). Fletcher (1920) isolated a mucoid form of *B. paratyphosus* B from two chronic ' carriers ', and Walker (1922) experimentally converted an ordinary strain of *B. paratyphosus* B into a mucoid variant by cultivation in an environment containing about 25 per cent. of specific immune serum.

Serology.

Agglutinins are developed in the blood of animals inoculated with the capsulated bacilli. As observed by Coulter (1917) and others the titre is low, often not exceeding 1 in 10 and seldom 1 in 60. If degraded strains that have been deprived of their capsule are employed much higher titres are obtained, but in this case type specificity is lost. Coulter noted that the addition of the bacteria to concentrated serum 1 : 1 or 1 : 5 dilution produced an almost immediate coarse flocculation settling as a compact disc to the bottom of the tube and leaving a clear supernatant fluid. As we shall see later the chief species, *B. pneumoniæ* of Friedländer, can by agglutination be classified into four chief types which are quite distinct in their immunization response. Bacilli deprived of their capsules, on injection into rabbits give rise to agglutinins not only for their own type, but for other types of the same species and for nearly allied species. The importance of the capsule in immunity has been clearly demonstrated, especially by the work at the Rockefeller Institute in recent years on *Pneumococcus* and *Pneumobacillus*.

Pathogenicity.

In man, Friedländer's bacillus is the primary infective agent in a small percentage of cases of pneumonia. It occasionally causes a generalized

septicæmia, but is most frequently found associated with inflammation of the nasal sinuses, antrum of Highmore and other parts of the respiratory passages. The bacillus of rhinoscleroma is found in the lesions of this disease and is considered by some to be the causative agent. *B. mucosus ozænæ* and *B. cyprinicida* are believed to be the cause of a fetid nasal catarrh in man and of a fatal disease in carp respectively.

The organisms of the group show a varying degree of pathogenicity to mice, guinea-pigs and rabbits, and Fricke (1896) and Clairmont (1902) attempted to classify the members according to their action on these animals, but such a classification did not conform with other characters of the members. Recent work has shown the importance of the capsule in determining the virulence of a strain, so that cultures of the same species show great differences, depending on their age, method of cultivation and other factors which affect the formation of capsules.

B. mucosus capsulatus.

Definition.

A Gram-negative encapsulated non-motile bacillus, characterized by a mucoid growth on agar; unable to produce indole and to liquefy gelatin, but capable of reducing nitrates and of forming ammonia and amino-acids from peptone, and of causing disease in man and other animals.

Differentiation from Allied Bacteria.

It is differentiated from the *Pneumococcus* group by its general cultural characters and by its being Gram-negative, but has in common with it the possession of a well marked capsule. In staining and in biochemical reactions, it is closely allied to the *B. coli* group, the *aerogenes* type being a connecting link. The organism was discovered by Friedländer in 1882, and was described by him as a coccus and as the incitant of lobar pneumonia. At first it was confused with what is now called the Pneumococcus of Fraenkel.

Morphology and Variation.

The chief characteristic of the bacillus is its plumpness and the roundness of its ends. Its width varies from 0·5 to 1·5μ and its length from 0·6 to 5μ. Forms which are almost coccal are met with and often predominate in blood, pus and sputum, and were responsible for Friedländer's description of the organism as a 'micrococcus'. The bacilli may grow out into long threads in media containing antiseptics and dyes, and on glycerin agar and potato. In films of cultures the bacilli may be seen single or in pairs and short chains. They are Gram-negative and are characterized by the possession of a capsule which is usually twice or three times the size of the bacillus.

Loss of capsule. In the course of growth on agar or gelatin capsule formation frequently ceases, and this degradation can be accelerated by cultivation at low temperatures (10° C.) or by artificial selection of the

smaller or dryer colonies for several generations. We shall see presently that the immunological reactions of the bacillus are profoundly altered when capsule formation is in abeyance. Such capsule-free strains have been found by Streit (1906), Beham (1912) and von Eisler and Porges (1906) to regain their mucoid condition on animal passage.

Staining characters. The bacilli are readily stained by the ordinary basic aniline dyes but are quite Gram-negative. The capsules can be demonstrated by the usual capsule stains.

Cultural Characters.

Temperature. For quickest growth 37° C. is the optimum temperature, but development readily occurs at 20° to 22° C., growth ceases below 10° C.

Reaction. A hydrogen ion concentration of pH 7 is probably the most suitable, but the bacillus is not much affected by slight deviations either to the acid or alkaline side.

Moisture. For the best development of a growth of a mucoid character on agar slants, potato, &c., the media should be freshly prepared and should contain an abundance of moisture.

Oxygen. The bacillus is aerobic and facultatively anaerobic, though in the absence of air the growth is not luxuriant.

On *agar* slants the characteristic mucoid growth occurs, the growth being raised, moist, slimy, and sometimes running towards the bottom of the tube, and presenting an appearance not unlike the gutterings on a candle. Single colonies are gray white, glistening, dome-shaped elevations on round regular bases, and when touched with a wire appear to be viscid.

Colony variation. Although as long ago as 1906, Streit, and subsequently Eisenberg (1914, 1918, 1919) and Baerthlein (1918), had observed among the moist colonies of Friedländer's bacillus, others which were drier and showed loss of capsule, it was only since Arkwright's (1921) work on ' smooth ' and ' rough ' strains of bacteria that their importance was fully appreciated. Julianelle (1926) from pure capsulated S strains obtained R non-encapsulated strains by cultivation in broth containing 10 per cent. of homologous immune serum, and then transplanting on to agar plates. From the dry colonies broth cultures were obtained which failed to kill white mice in doses of 0·5 c.cm., while the parent S strain from which the R had been derived regularly killed within 48 hours at a dilution of one ten-millionth c.cm.

Hadley (1927) describes in connection with the colonies of Friedländer's bacillus and of various intestinal bacteria what he terms 'marginal dissociation', consisting of the appearance of peripheral, bluish, translucent invaginations and fringes composed almost exclusively of the R type of culture.

Action of selective substances, e.g. dyes. In relationship to bile salts, dyes, &c., Friedländer's bacillus behaves in the main like *B. coli.* Fitzgerald (1914) found gentian violet, by suppressing the growth of

Gram-positive cocci, useful in the isolation of encapsulated bacilli. He found differences in various strains of Friedländer's bacillus in their sensitiveness to the dye.

Gelatin. In stab cultures the growth on the surface is sometimes raised in a dome-shaped fashion and has been compared to a nail head. This appearance depends greatly on the amount of moisture in the medium ; a somewhat dry medium favours its development. Along the track of the stab growth occurs and may show feathery outgrowths ; no liquefaction takes place. Development of a brownish colour in gelatin after long periods of growth and also in glycerin agar, was found by Perkins (1904) to be variable and inconstant. In bouillon there is a uniform turbidity with formation of a thin pellicle if the tubes are not disturbed ; a heavy stringy deposit is found.

On *potato,* Fricke (1896) believed that the appearance of the growth of different members of the group showed such variation that the medium might be employed for the purposes of classification. Clairmont (1902) and others were unable to confirm his views. In general an abundant moist growth of a brownish colour develops.

In *peptone* solutions there is uniform turbidity and no indole formation.

Litmus milk. In this medium there is abundant growth and well-marked capsule development. Acid is usually produced and frequently coagulation occurs. Small and Julianelle (1923) found that in litmus milk after one week, 3 strains produced acid, coagulation and the beginning of peptonization ; 16 strains produced acid sufficient for coagulation ; 4 strains showed only slight acidity, and 4 showed no visible change.

Methods of primary isolation. Sputum, pus or other material should be planted on the surface of agar or on Conradi-Drigalski plates and the study of mucoid colonies pursued.

Viability and Lytic Phenomena.

The bacillus has much the same longevity under various conditions as *B. coli.*

Caublot (1924) obtained from the stools of a patient who had recovered from a septicæmia caused by Friedländer's bacillus, a bacteriophage which caused typical transmissible lysis with formation of characteristic plaques in the growth on agar.

Biochemical Reactions.

Proteins. Friedländer's bacillus is unable to liquefy gelatin or to digest coagulated serum or albumin. It is, however, capable of forming ammonia and amino-acids but not creatinine from peptone. Small and Julianelle (1923) noted inability to form indole in all their strains, a result substantially in agreement with that of Clairmont (1902), but differing from those of Perkins (1904) and Fitzgerald (1914).

Carbohydrates. The bacillus has a strong fermentative action on many carbohydrates. The findings of Small and Julianelle (1923), who

employed 18 carbohydrates in their examination may be summarized as follows. The majority fermented, with or without the formation of gas, dextrose, lævulose, maltose, rhamnose, arabinose, xylose, adonitol, mannitol, glycerin. About one half fermented salicin, very few fermented dextrin, starch and dulcitol, and none fermented inulin. They found that the action on dextrose, lactose and saccharose differed in different strains, and their results were not in complete accord with those of Clairmont (1902), Perkins (1904) and Fitzgerald (1914).

On the ground of their action on dextrose, lactose and saccharose the strains were placed by Small and Julianelle in four divisions. (1) Strains which fermented dextrose, lactose and saccharose with formation of gas, 8 strains ; with or without formation of gas, 5 strains. (2) Strains which fermented dextrose and lactose but not saccharose with formation of gas, 3 strains ; with or without formation of gas, 5 strains. (3) Strains which fermented dextrose but not lactose or saccharose with formation of gas, 2 strains ; with or without formation of gas, 2 strains. (4) Strains which fermented neither dextrose, lactose nor saccharose, 2 in number. These groups were constituted by granuloma and respiratory strains alike. Small and Julianelle concluded that the carbohydrate fermentation reactions were irregular and unreliable as a means of identification or of subgrouping the strains. In this they were in agreement with Fitzgerald (1914) who ended his paper with the words : ' So far our work cannot be taken to support any grouping of the Gram-negative encapsulated bacilli hitherto proposed. Such divisions have been based on differences in staining reactions, cultural features, biochemical activities or pathogenicity. After a careful review of these points in the cultures studied by us, it does not seem possible at the present time to constitute species on the basis of differences shown. It seems more than likely that this group is most closely related to the colon, the essential point of distinction being the possession of a capsule. It is conceivable that mutations based on the necessity of maintaining a parasitic existence have caused Gram-negative bacilli found normally in the body elsewhere than in the intestinal tract, to develop capsules for protection, and a new group has arisen which we designate *B. capsulatus mucosus,* and the varieties *B. aerogenes* and *B. acidi lactici* connect the group with the non-encapsulated Gram-negative bacilli belonging to the colon group '.

Small and Julianelle (1923) found the Voges-Proskauer and the methyl red reactions of no value in classification. In general there was a greater tendency towards the production of a positive methyl red reaction than towards the production of a positive Voges-Proskauer, but no strict correlation between the two tests was evidenced. Two strains studied by the same authors derived from lesions unrelated to rhinoscleroma failed to produce gas in any of the carbohydrates fermented, and in this respect resembled *B. rhinoscleromatis,* so that inability to produce gas cannot safely be considered a differential character of *B. rhinoscleromatis.*

Serological Reactions.

It was noted by numerous workers that though agglutinins developed in the blood of animals inoculated with encapsulated bacilli, the titre was exceedingly low. This led to efforts being made to increase the titre by using as antigen cultures deprived of their capsules by growth at low temperatures and by selection of dry, or, as we now term them, R, colonies. Julianelle (1926) took up the question, being influenced by the principles governing the immunological relationship of pneumococci and their cell constituents—principles which had been worked out by his colleagues at the Rockefeller Institute of Medical Research. According to this newer concept the bacterial cell contains two separable and distinct constituents —the one a polysaccharide, the soluble specific substance which endows the cell with type specificity; the other a protein substance, which, regardless of type derivation exhibits immunologically only the common and undifferentiated characteristics of the species. The dissociation of the polysaccharide from the cell deprives the organism of its type-specific antigenic power, hence, in this case, degraded unencapsulated strains lose the property of stimulating the type-specific antibody, and as antigen provoke only the common protein response—a response shown by action not only on the protein of Friedländer's bacillus, but also on that of *B. rhinoscleromatis*, *B. aerogenes*, typhoid and granuloma bacilli. Using, therefore, only capsulated bacilli for his antigen, Julianelle (1926) found that among 30 strains of Friedländer's bacillus, there were 3 sharply defined and specific types and 1 heterogeneous group. To type A, 15 strains; to type B, 6 strains; to type C, 3 strains; and to Group X, 6 strains were assigned.

The soluble Specific Substance of Friedländer's Bacillus.

The observation of a specific soluble substance in *Pneumococcus* by Dochez and Avery (1917) and its identification by Heidelberger and Avery (1923) with the polysaccharide portion of the organism, suggested that the polysaccharide already isolated from Friedländer's bacillus by Toenniessen (1921) and Kramár (1921) would also possess specific properties. The work of Müller, Smith and Litarczek (1924–5), and of Heidelberger, Goebel and Avery (1925) confirmed this assumption. They found that this polysaccharide caused a precipitate in high dilutions—1 in 2 million—of a homologous immune serum. Heidelberger, Goebel and Avery (1925) describe the soluble specific substance isolated by them from a strain of Friedländer's bacillus as a white fluffy amorphous powder with acid properties, sparingly soluble in water when dry, but yielding soluble alkali salts. The polysaccharide, which was nitrogen-free, had an optical rotation of + 100, was itself non-reducing, but on hydrolysis yielded reducing sugars, among which glucose was present. It showed in its characters a close relationship to the specific soluble substance of Type II *Pneumococcus*, and both substances showed themselves reciprocally reactive with the antibacterial serum of each in a dilution of 1 in 2 million.

Protection experiments also demonstrated the similarity of the two substances, since mice inoculated with 0·2 c.cm. antipneumococcus serum Type II were protected against at least 1,000 lethal doses of the virulent E strain of Friedländer's bacillus, and similarly anti-Friedländer serum protected against Type II *Pneumococcus*. They believed that, as in *Pneumococcus*, the polysaccharide by itself was not antigenic, but would become antigenic when attached to some other substance, possibly the protein of the cell. This was demonstrated to be the case by the later work of Julianelle (1926), who showed that although antigenically the polysaccharide of Friedländer's bacillus was inert, yet in the form in which it existed in the cell, the carbohydrate complex was the dominant and effective antigen, yielding on immunization the corresponding type-specific antibody. He also pointed out that cell dissolution, whether occurring spontaneously in the body or artifically *in vitro* is accompanied by more or less antigenic dissociation, and that the result of this process is reflected in the immune serum by the increasing amount of non-specific antibody with diminution or complete loss of type-specific response. Such serum of high titre agglutinates not only Friedländer's bacillus but also a number of organisms, which, however, are unable to remove agglutinins of the specific type by absorption of the serum. Evidence of this dissociation of the components of the bacilli in the body is given by the demonstration of the soluble specific substance of Friedländer's bacillus in the blood and urine of man (Blake, 1918) and of animals (Julianelle, 1926) infected with it.

Immunological Reactions of 'Rough' and 'Smooth' Strains.

Following the nomenclature of Arkwright (1921), Julianelle (1926) designates the ordinary capsulated colonies of Friedländer's bacillus 'S', and the dry, rough, non-encapsulated colonies 'R'. In his study the criteria he applied for the conversion of S organisms to R forms were: (1) loss of capsule and mucoid characteristics; (2) loss of agglutinability in type-specific sera; (3) attenuation of virulence; (4) the development of colonies which present, under the microscope, a rough and irregular surface. As a result of his experiments, Julianelle concluded:

(1) The S strains of Friedländer's bacillus produce capsules and soluble specific substance, and are of exalted virulence. S strains are type-specific and react with only the type-specific antibodies of the homologous types.

(2) Immunization with S cells induces the formation of antibodies which agglutinate type-specifically, precipitate the corresponding soluble specific substance and protect white mice against infection caused by organisms of the same type. R strains of Friedländer's bacillus produce no capsules, produce no soluble specific substance and are not pathogenic. R strains are serologically undifferentiated from each other and react with only the species antibodies.

(3) Immunization with R cells induces antibodies which do not agglutinate encapsulated organisms, do not precipitate soluble specific substance,

and do not afford protection against infection by Friedländer's bacillus. Anti-R serum contains only the species antibody which reacts with any capsule-free organism regardless of its type origin.

(4) Decapsulation of S cells by heat and acid chemically converts a type-reacting organism into a species-reacting organism.

Pathogenic and Antigenic Action in Animals and Man.

Animals. Cultures of Friedländer's bacillus are pathogenic to mice, guinea-pigs and rabbits when injected subcutaneously, intravenously and intraperitoneally. Spontaneous infections of guinea-pigs by this bacillus are described by Branch (1927). Fricke (1896) and Clairmont (1902) attempted a classification of the encapsulated bacilli according to their pathogenicity for the three common laboratory animals. Inoculation is followed by local inflammation and death by septicæmia, and the exudates are characterized by a stringy mucoid nature. No evidence is forthcoming that the animals can be infected by the presence of bacilli in their food.

Analysis of pathogenic action. No extracellular toxin has been found in cultures. Julianelle (1926) demonstrated the presence of the specific soluble substance in cell-free filtrates of cultures of Friedländer's bacillus as early as four hours after growth has been initiated, it being evidently a product of metabolic activity and not of cell disintegration. It was not toxic and by itself was not antigenic. It was only after nine days' growth that protein appeared in the filtrate, due, doubtless, to autolysis. This protein was antigenic, but was not toxic in its action. In the section on serology attention has been directed to the importance of the capsule in determining virulence.

Dose. Encapsulated bacilli in broth culture have been found by Julianelle and others to kill mice when inoculated intraperitoneally in amounts of ·0000001 c.cm., whilst unencapsulated strains in doses of 0·5 c.cm. are without effect.

Pathology and serology in man. Friedländer's bacillus is the infective agent in only a small percentage of cases of pneumonia ; Weichselbaum puts this percentage at 7 or 8, but this is probably too high. Such cases are extremely severe and usually fatal. It has also been found in bronchitis, in inflammation of the antrum and the nasal sinuses ; in serous cavities, e.g., pleurisy, pericarditis and peritonitis ; in cystitis, pyelonephritis and in endometritis ; in otitis media and in meningitis, usually subsequent to inflammation of the middle ear or nasal sinuses. Howard and Blumer have described cases of general infection in which the septicæmia was of a hæmorrhagic type.

Schmidt (1903) at the end of a month observed the appearance of agglutinins and bacteriolysins for Friedländer's bacillus in the serum of a patient suffering from pneumonia. Wolf (1908) in a case of cystitis found the titre of the serum for the homologous Friedländer's bacillus, and for several stock laboratory strains to be 1 in 2,000 to 1 in 5,000.

Mechanism of Spread of Infection.

Outside the animal body Friedländer's bacillus has been found in the soil, in dust and in water. It is occasionally found in the nose and throat of healthy men. It is also met with in the respiratory passages of rabbits and guinea-pigs. Perkins (1900–1) found that guinea-pigs which survived after a spontaneous Friedländer epidemic had acquired immunity against the organism isolated during the course of the disease. Where infection of men and animals occurs it may be due to the inhalation of a virulent strain, or a strain normally saprophytic in the body may have been able through some lowering of the natural resistance of the body by exposure, fatigue, &c., to attack.

Detection of the Bacillus.

In sputum and pus the appearance is quite characteristic on examination of films stained by Gram's method and counter-stained with fuchsine—Gram-negative capsulated bacilli. Cultures can readily be obtained on Conradi-Drigalski medium. In a generalized infection blood culture is necessary for a bacteriological diagnosis.

Other Members of the Mucoid Encapsulated Group.

Bergey (1926) includes in the genus *Klebsiella* Trevisan six species, to which he gives the following key :

1. Acid and gas in dextrose, lactose and saccharose :
   (*a*) Nitrates reduced to nitrites :
      (1) *Klebsiella pneumoniæ.*
      (2) *Klebsiella granulomatis.*
2. Acid and gas in dextrose and sometimes in lactose :
   (*a*) Nitrates reduced to nitrites :
      (3) *Klebsiella rhinoscleromatis.*
      (4) *Klebsiella capsulatus.*
   (*aa*) Nitrates not reduced :
      (5) *Klebsiella ozænæ.*
3. No acid or gas in carbohydrate media :
      (6) *Klebsiella ciprinicida.*

We have already seen that the sugar reactions are not sufficiently constant to form the basis of a classification of the group, so that this attempt of Bergey's is far from satisfactory. However, in the absence of anything better, it is perhaps not without value. We shall now refer briefly to a few of the members.

Bacillus of Rhinoscleroma (*syn.* Klebsiella rhinoscleromatis).

Rhinoscleroma is a slowly developing granulomatous inflammation at the external nares or upon the mucosa of the nose, mouth, pharynx or larynx. In large swollen cells, ' Mikulicz cells ', lying amongst the connective tissue and in the intracellular spaces of the granuloma, the bacilli of rhinoscleroma can be demonstrated on histological examination.

This bacillus was described by Frisch in 1882, and Wilde (1896) thought that by forming no gas in dextrose bouillon and in producing no acid in lactose bouillon and in never coagulating milk it could be distinguished from Friedländer's bacillus, to which it presents the greatest resemblance. We have already pointed out that Small and Julianelle (1923) found Friedländer strains possessing the above fermentative characteristics.

The ætiological relationship of the bacillus to the disease is by no means definitely established. The bacillus probably belongs to one of the types of Friedländer's bacillus. Goldzieher and Neuber (1909), using serum from patients suffering from the disease and from rabbits immunized to the rhinoscleroma bacillus, found fixation of complement with an antigen consisting of *B. rhinoscleromatis*, but not with one composed of Friedländer's bacillus. Other workers, e.g. Erben (1906), Ballner and Reibmayr (1907) and Galli-Valerio (1910), found no serological difference between the two organisms.

The presence of a complement-fixing antibody for *B. rhinoscleromatis* in the sera of rhinoscleroma patients has been demonstrated by numerous investigators and is strong evidence that the bacillus is at any rate more than a mere saprophyte. For recent work on the subject, the papers of Elbert, Feldmann and Gerkes (1925) and Quast (1926) may be cited. Quast found that complement fixation occurred with the patient's sera, only with an antigen consisting of *B. rhinoscleromatis*, not with one consisting of the Friedländer or ozæna bacilli.

Bacillus mucosus ozænæ *Abel* (*syn.* Klebsiella ozænæ [*Abel*] *Trevisan*).

This bacillus was cultivated by Abel in 1893 from the nose in cases of ozæna, and was believed by him to be the infective agent in this condition. Culturally, it is very similar to Friedländer's bacillus, and its growth is not accompanied by any offensive odour. It is doubtful whether *B. mucosus ozænæ* has any ætiological connection with the disease, although Abel (1928) still regards it as the primary cause of the ' ozæna process ', the peculiarity of which is the formation of a tenacious, slimy, purulent secretion which dries and forms scabs on the surface of the mucous membrane. According to Abel, the process may or may not be associated with a disagreeable odour, and the atrophy of the turbinate bones is only an end result.

Most authorities now regard *B. mucosus ozænæ* as a type of the Friedländer group. Michailoff (1926), after a comprehensive review of the literature dealing with the bacteriology of ozæna and after a very full study of the cultural, serological and pathogenic action of the Proteus-Perez group of micro-organisms, concludes that the bacillus isolated by Perez (1899) is closely related to *B. proteus*, and that the *B. ozænæ liquefaciens* isolated by Shiga (1922) is a typical *B. proteus vulgaris*, and that these micro-organisms are involved in the pathogenesis of ozæna and whether implanted primarily or secondarily are the cause of the fetor, discharge and ulceration. Michailoff found considerable variation in the

cultural character of various strains of the *Coccobacillus fœtidus ozænæ* of Perez. In the main, the following description applies to most of the members: They are Gram-negative, non-motile, non-capsulated, non-sporing bacilli which grow well on ordinary laboratory media, may or may not liquefy gelatin, but all form indole and produce a putrid odour in broth and on agar plates; all ferment lævulose, all produce gas and fluorescence in neutral red dextrose agar; none ferment lactose, raffinose, inulin, dextrin, starch, dulcitol or mannitol; none produce hæmolysis in agar plates to which rabbit whole blood or washed red cells have been added.

Bacterium cyprinicida *Plehn* (*syn.* Klebsiella ciprinicida [*Plehn*] *Bergey*, et al.).

In its growth on the usual media this bacillus, which was isolated by Plehn (1903–4) from a fatal disease in carp, resembles Friedländer's bacillus, but unlike most strains of that bacillus it produces no acid in carbohydrate media. The optimum temperature for its growth lies between 10° C. and 20° C.

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The B. alcaligenes Group.

The Group as a Whole.

Definition.

Short Gram-negative bacilli usually occurring in the intestinal tract, which do not form acetyl-methyl-carbinol and do not ferment any of the carbohydrates.

Characters.

Form: rods, 0·5 by 1 to 3μ, usually motile by means of peritrichous flagella, non-sporing and not capsulated, Gram-negative. *Fermentations:* alkali instead of acid formed in carbohydrate media. *Gelatin:* gray or translucent growth, usually no liquefaction. *Agar:* flat or raised moist growth. *Potato:* growth usually moist, raised and brownish. *Milk:* alkaline, no clotting. *Broth:* turbid and often with pellicle, indole not formed. *Optimum temperature* for growth 37° C.

On the basis of gelatin liquefaction, nitrate reduction and motility Bergey (1926) subdivides the members of the group thus: gelatin is not liquefied by *B. fæcalis alcaligenes* and *B. bronchisepticus*, which are both motile, but differ in that *B. fæcalis alcaligenes* is able and *B. bronchisepticus* is unable, to reduce nitrates to nitrites. Bergey also considers *Bacillus abortus* of Bang and *Micrococcus melitensis* as non-motile and non-reducing members of this group. These latter organisms will not be described in this section.

Of the gelatin-liquefying members, Bergey mentions four, two of which, *B. alcaligenes bookeri* and *B. alcaligenes recti*, cause saccate liquefaction,

while the shapes of the liquefaction produced by *B. alcaligenes marshalii* and *B. alcaligenes albus* are infundibuliform and stratiform respectively.

The Relationship of the Group and its Members.

In morphology and the character of the growth on ordinary media, the members of this group resemble those of the coli-typhoid group, but they differ in the complete absence of sugar-splitting enzymes. *B. bronchisepticus*, we shall see later, is said to be closely related serologically to *B. pertussis*. *B. abortus* and *B. melitensis* resemble the group in the appearance of their growth in litmus milk, and on potato, and in their inability to ferment sugars; morphologically they are smaller and are non-motile.

B. fæcalis alcaligenes.

Definition and Description.

B. fæcalis alcaligenes (syn. *Alcaligenes fæcalis*) is a Gram-negative motile bacillus unable to ferment any carbohydrate, producing a strong alkaline reaction in litmus milk, and a brownish growth on potato, frequently found in the intestinal canal, and very occasionally infecting its host. This bacillus was observed by Petruschky in spoiled beer in 1889, and a description of its chief characters was given by this observer in 1896. It usually has the significance of a simple saprophyte, but accounts have been given by a number of observers which indicate that it may cause an infection of the body of man.

Morphology and Staining Reactions.

All observers agree in describing *B. fæcalis alcaligenes* as a motile Gram-negative bacillus. The size and shape of the organism varies a little according to the conditions under which it is grown, but in general, it may be described as a short rod, 0·5 by 1 to 2μ, occurring singly or in pairs, and occasionally in long chains and provided with peritrichous flagella. Baerthlein, Glaser and Hachla (1911) and Pollak (1913) found that on Dieudonné's medium the morphology of the bacillus resembled that of the cholera vibrio. Most observers describe it as peritrichous, but Klimenko (1907) found that the flagella were polar, 2–4–6 occurring at both ends, and Kühnemann (1911) also found flagella not along the sides, but usually confined to one pole and numbering one or several. Bergey in his classification, definitely states that the type species is peritrichous.

Cultural Characters.

The bacillus closely resembles *B. typhosus* in its growth, and flourishes on similar media. This similarity of growth has sometimes led to difficulty in the isolation of *B. typhosus*. On the Conradi-Drigalski medium the appearance of the colonies of the two organisms is very similar.

On *agar* slopes the growth is moist, slightly raised, and slightly bluish when examined by transmitted light. When broth cultures were planted on the surface of agar plates, Felsenreich and Trawinski (1916) found

among their strains two varieties of colony ; one was flat and transparent, a second opaque, dull and raised. According to these observers, these varieties bred true to type. The two types of colony were best marked on the Conradi-Drigalski medium, on which Trawinski and György (1918) studied 100 different strains, and of these 69 and 31 formed raised and flat colonies respectively.

On *gelatin* the growth is similar to that of *B. typhosus* and no liquefaction of the medium occurs. Petruschky and all subsequent observers have laid stress on the value of potato and litmus milk as diagnostic media.

The growth in *litmus milk* is accompanied by the prompt production of alkali, the medium becoming dark blue in colour.

In *ordinary milk*, after 10 days' incubation, a yellow colour develops, but clotting is absent even after 28 days at 37° C.

In *peptone water* there is after 24 hours' growth a uniform turbidity and the formation of a very slight deposit. No pellicle is found even after prolonged cultivation.

In *broth*, Trawinski and György (1918) confirmed in the main Gaehtgens's (1907) description. They found the turbidity of broth to be accompanied by the formation of a thick pellicle within 24 hours in the majority of their strains. In a few, the pellicle was only found after a growth of 36, 48 or 72 hours, or even in some instances of 4 to 6 weeks. They found that the pellicle of those strains that formed it early behaved as Gaehtgens described, i.e. it fell *en masse* to the bottom of the tube on shaking. On the other hand, pellicles of later development might either sink *en masse* or break up into flakes in the process. No smell is described as occurring on any medium.

On *potato* typical strains of *B. fæcalis alcaligenes* produce an abundant moist brown growth, but Kraus and Klaften (1918) describe closely allied varieties producing only scanty growth. On blood-alkali agar plates (Dieudonné) the growth is similar to that of the cholera vibrio (Glaser and Hachla, 1911 ; Pollak, 1913 ; Trawinski and György, 1918).

Biochemical Reactions.

Carbohydrates. No observer has described the fermentation of any carbohydrate. Trawinski and György (1918) obtained negative results with dextrose, lævulose, galactose, mannose, saccharose, lactose, maltose, raffinose, inulin, starch, glycerin, erythritol, xylose, arabinose, rhamnose, dulcitol, mannitol, sorbitol, and inositol.

Proteins. No proteolytic ferment is formed and no digestion of serum, gelatin or casein occurs, although the growth of the organism is accompanied by the formation of alkali. No indole is formed. Nitrates are reduced to nitrites.

Serological Reactions.

In the blood-serum of man, in whose intestine, especially in enteric fever, the bacillus may be present in large numbers, no agglutinins seem

to be developed. Trawinski and György (1918) studied, at intervals, the action of the sera of 31 cases of enteric-like conditions not only on the typhoid-paratyphoid group, but also on *B. fæcalis alcaligenes*. Only in one case did agglutination occur in 1 in 50 or higher dilution, and the agglutinins in these cases were proved by a saturation experiment to be mitagglutinins produced by the action of *B. paratyphosus* B. It is worthy of note that in cerebrospinal fever cases Symmers and Wilson (1908) and Wilson (1909) observed agglutination of a bacillus of the *B. fæcalis alcaligenes* class which they had isolated from water and designated *B. aquatalis alcaligenes*. No explanation for the development of the heterologous agglutinins was forthcoming. A few cases are on record of infection with *B. fæcalis alcaligenes* with the production of agglutinins in the blood. Such are those of Shearman and Moorhead (1916), Hirst (1917), de Magalhaes (1921), and Rochaix and Marotte (1916).

Rabbits inoculated with *B. fæcalis alcaligenes* produce agglutinating serum of high titre. Klimenko (1907) and Gaehtgens (1907) in a study of several strains of the *B. fæcalis alcaligenes* group found that the serum prepared for any one strain did not agglutinate all, but only a few of, the other strains. They believed that as regards agglutinins there were the same differences in the *B. fæcalis alcaligenes* group as existed in that of the *B. coli*. The results of Trawinski and György (1918), however, suggest that there is far more uniformity than previous observers suspected. We have already referred to the different types of colony described by Felsenreich and Trawinski (1916), and it is interesting that, according to Trawinski and György (1918), the grouping of the strains by the nature of the colony and by the agglutination test were absolutely correlated. Of their 100 strains they found that 69 which formed raised dome-shaped colonies, were agglutinated to full titre 1 in 12,000 by the serum of a rabbit which had been inoculated by one of them, and that this serum had little or no action on 31 strains which formed flat colonies. On the other hand, a serum prepared for one of the flat-colony-forming strains agglutinated it and the other 30 similar strains to full titre (1 in 10,000).

Pathogenicity.

Petruschky was able to infect laboratory animals by intraperitoneal, but not by subcutaneous inoculation. Trawinski and György (1918) also inoculated white mice subcutaneously with negative results.

Although *B. fæcalis alcaligenes* is frequently found in man's intestine, and may be found in large numbers in cases of enteric fever, it is rare to find any evidence of its infectivity. Some regard it as a pure saprophyte. During the war in spite of the enormous numbers of blood cultures made in enteric-like conditions, hardly ever was *B. fæcalis alcaligenes* encountered. Trawinski and György (1918) in thousands of examinations had negative results. A study of the literature shows that in a few cases of diseases resembling enteric fever *B. fæcalis alcaligenes* has been cultivated from the blood. For instance, Laforgue (1908), Straub and

Krais (1914), Rochaix and Marotte (1917), Shearman and Moorhead (1916), Hirst (1917) and de Magalhaes (1921) make such reports.

A description of a group of cases of a mild paratyphoid-like fever, which occurred at one hospital in Egypt and in which *B. faecalis alcaligenes* was repeatedly isolated from the blood, is given by Shearman and Moorhead (1917). Ledingham (1923) refers to a similar small group of 10 cases in Mesopotamia at Amara, where from 4 of the 10 *B. faecalis alcaligenes* was recovered from the blood. Petruschky (1902) isolated the bacillus from spots in the skin of a typhoid patient, whilst Fischer (1899) found the bacillus present in pure culture in the organs of a patient who suffered from tuberculosis and broncho-pneumonia, and Hamm (1910) found it present in a patient with pyelonephritis of pregnancy who eventually developed puerperal endometritis and peritonitis.

It would seem, however, that evidence of this organism being pathogenic to man or other animals is very rare.

Distribution in Nature and Man.

Petruschky originally found his bacillus in spoiled beer. The writer has frequently met with strains of it in water. It is probably very widely distributed in nature, and is a mere saprophyte. In the human intestine under normal conditions it probably occurs only in small numbers. Trawinski and György (1918), in the examination of the stools of 386 healthy soldiers, found the bacillus 73 times, i.e. in 19 per cent. On the other hand, in the examination of the stools of 126 soldiers suffering from a catarrhal condition of the bowel, the bacillus was found in 90, i.e. in 71·5 per cent. An inflammatory condition of the intestine thus provides conditions favourable to growth, but there is no evidence that as a result infection occurs.

Detection of the Bacillus and Maintenance of Cultures.

B. fæcalis alcaligenes grows on the ordinary laboratory media, and has a viability corresponding to that of *B. coli*. For its isolation the Conradi-Drigalski medium alone suffices, but we have already referred to the fact that it grows well on Dieudonné alkali blood-agar medium, and this medium has been found very useful for its isolation. Trawinski and György (1918), by adding 2 or 3 *per cent.* to peptone water, and incubating for 6 to 8 hours and then subculturing on Dieudonné's medium, obtained 80 per cent. more successes than when they planted out the stools directly on to Conradi-Drigalski plates.

B. bronchisepticus.

Definition, Description and Significance.

A Gram-negative, actively motile bacillus unable to liquefy gelatin or coagulated serum or to ferment any carbohydrate, but capable of producing a spreading tan-coloured growth on potato. Ferry (1910 and 1911) and independently McGowan (1911) described this bacillus. Ferry at first

named it *B. bronchicanis*, and later *B. bronchisepticus* on recognizing its presence in epizootics among laboratory animals other than dogs. Both observers regarded it as the primary infective agent in distemper of dogs. Canine distemper was also thoroughly studied by Torrey and Rahe (1913) who had commenced their work and had isolated a bacillus identical with *B. bronchisepticus* before Ferry's and McGowan's papers were published. McGowan isolated it also from a man, a monkey, several guinea-pigs and rabbits, and from numerous cats and dogs. It is very doubtful if it is to be regarded as the cause of the disease. A certain amount of evidence has been furnished both for and against the view of Carré (1905) that ' distemper ' is caused primarily by a filterable virus, and that the disease as a whole consists of a series of progressive secondary infections by a number of bacteria (see Vol. VII).

Morphological, Cultural and Biochemical Characters.

The bacillus is a short slender rod, 0·4 to 0·5 by 2·0μ, usually found single, but often in pairs. In liquid media and in the tissues it may be found in chains. In the tissues the organism has often a more or less coccal form and this shape may be found in cultures made on agar from them.

Staining reactions. It is Gram-negative, non-sporing, and, when stained with Löeffler's methylene blue, has a characteristic bi-polar appearance.

Cultivation. On the surface of plain *agar* a culture from tissues appears after 20 hours as a number of discrete dew-points which enlarge very rapidly during the next 24 hours, becoming as large as pins' heads. They are raised above the surface, are regular hemispheres in shape, have an opaque white porcelainous look with an opalescent sheen. The spreading growth is smooth, glistening and sticky, without odour at first, but in old culture a stale or musty smell is detected. Although *B. bronchisepticus* is not an obligate aerobe it thrives very poorly in absence of oxygen. In an *agar* stab the growth is filiform and better near the surface. In *gelatin* the appearance is similar to that in agar and no liquefaction occurs.

All observers are agreed that the two media of the greatest importance in the identification of *B. bronchisepticus* are litmus milk and potato. In *litmus milk*, the growth of *B. bronchisepticus*, like that of *B. fæcalis alcaligenes*, is accompanied by a progressive change to intense alkalinity. The alkalinity begins at the surface, and proceeds downwards, the change being evident in 24 hours. In 5 to 10 days the whole medium has assumed a blue-black colour. After 15 days the lower fourth of the tube is bleached, but even after several weeks the bleaching does not extend beyond the lower third. In plain milk there is no obvious change up to 4 days, when the medium becomes slightly translucent. This translucence becomes marked in 7 days, and in 22 days the medium is changed to a clear yellow watery fluid with a slight sediment.

On *potato* after 24 hours at 37° C. the growth is abundant, moist, raised, glistening, and of a light tan colour. In 2 or 3 days it becomes

typically raised, moist, and the colour that of copper or Van Dyck brown, whilst the potato itself becomes dark brown. The odour is musty like that of stale bread. The growth is more raised and moister than that of *B. fæcalis alcaligenes* on this medium.

On *coagulated serum* there is a moist glistening growth which slowly becomes brown, but no digestion of the serum occurs. In *broth, glucose broth* and *peptone water* there is in 24 hours, turbidity, slight deposit, and no pellicle. After 72 hours a greyish growth appears on the sides of the tube at the surface and hangs down in tendrils into the fluid, and a rather viscid sediment is formed. No indole is produced.

Fermentations. There is no acid or gas developed in peptone water or broth containing glucose, galactose, maltose, saccharose, lactose, raffinose, mannitol, dulcitol, salicin, adonitol, inulin and inositol. According to Bergey (1926) nitrates are not reduced, but ammonia is formed from urea and asparagin. Torrey and Rahe (1913), however, found that although the great majority of their strains did not act on nitrates, a few did, and that this cultural reaction was correlated with morphological difference. Torrey and Rahe designate these forms, type A and type B, the latter reducing nitrates and being decidedly coccoid in shape especially when cultivated on glycerin agar.

Hæmolysis. Torrey and Rahe find that the *B. bronchisepticus* exerts a strong hæmolytic action on dog, rabbit or guinea-pig erythrocytes, when these are incubated in nutrient agar and the hardened surface has been inoculated. At the end of 24 hours there is good growth but no hæmolysis, but after 48 hours a clear band appears on each side of the streak which continues to spread for five days.

Action of Chemical and Physical Agents.

Heat. McGowan found that exposure of emulsions of *B. bronchisepticus* for one half-hour at 50° C., resulted in the death of the bacteria.

Desiccation. Torrey and Rahe report that the growth scraped from serum-agar cultures, dried over calcium chloride, and mixed with stale dust contained living organisms after three, and, in one instance, after five months' storage when kept in the dark. At the end of six months all the bacilli had died.

Cold. Torrey and Rahe found that broth cultures were alive after 29 days' exposure to the cold of winter, in spite of the fact that during 24 days, the mean temperature had remained at 0° C. or below, with frequent periods below −12° C., and at one time −19·5° C. After 43 days under these conditions, the bacilli were all dead.

Light. Torrey and Rahe report that bacteria suspended in salt solution and smeared over sized writing paper and then dried and exposed to sunlight were killed after 15 minutes or earlier. In broth cultures, McGowan had found the bacilli alive after 11 months' exposure to the light from the windows of his laboratory.

Chemical germicides. Torrey and Rahe found *B. bronchisepticus* very sensitive to the action of mercuric chloride, a 1 in 100,000 solution killing in less than five minutes when two large loopfuls of a 24-hour broth culture were added to equal amounts of the disinfectant solution. Under similar conditions, a 1 in 200 solution of lysol killed in 10 minutes, a 1 in 100 phenol in 5 minutes, and alcohol in strengths varying from 30 to 100 per cent. killed in less than 5 minutes.

Serological Reactions.

Agglutinins. Ferry (1911) found that the serum of dogs suffering from distemper agglutinated *B. bronchisepticus* in dilutions ranging from 1 in 40 to 1 in 800. The various strains employed by him were agglutinated to approximately the same titre. The serum of a dog immunized by repeated injections of one strain agglutinated it and four other strains in a 1 in 2,000 dilution and a fifth strain in a dilution of 1 in 600. The various strains would appear to be closely related serologically. McGowan (1911) also observed that in an immunized animal the serum generally has marked agglutinative properties for strains of the organism other than that with which the animal had been inoculated. Ferry and Noble (1918) showed that a close relationship, both culturally and serologically, existed between *B. bronchisepticus* and *B. pertussis.* The serum of a rabbit injected with *B. bronchisepticus* agglutinated this micro-organism in a dilution of 1 in 6,400, and 14 strains of *B. pertussis* in dilutions of 1 in 800 to 1 in 2,000. When the *B. bronchisepticus* serum was absorbed with *B. bronchisepticus* sufficiently to remove its own agglutinins, the agglutinins for *B. pertussis* remained unaffected. For the fixed agglutinins, the writers proposed the term ' transitive ' agglutinins. On the other hand they found that specific *B. pertussis* serum had no agglutinative action on the strains of *B. bronchisepticus* tested.

Immune body. Ferry and Klix (1918) confirmed the close relationship of *B. bronchisepticus* and *B. pertussis* by complement-fixation tests, finding that the *B. bronchisepticus* immune serum bound the complement in the presence of both the *bronchisepticus* and *pertussis* antigens, while the *B. pertussis* immune serum bound the complement in the presence of the homologous antigen and also of that of the human and monkey strains, but not of the dog strain of *B. bronchisepticus.*

Pathogenicity.

Ferry (1911) believed that his experiments proved that *B. bronchisepticus* was the cause of canine distemper, since he found the bacilli in the respiratory tract of every dog suffering from the disease, and also demonstrated the presence of agglutinins in their sera. Moreover, he stated that he produced typical distemper by artificial inoculation with pure cultures, the bacilli being again isolated and grown in pure culture and all under conditions which precluded infection from any other source. Torrey and Rahe (1913) reached similar conclusions. McGowan (1911), whose work was done independently and about the same time as that

of Ferry was more guarded, but concluded that he had produced a fair amount of evidence that the organism in pure culture can produce in healthy dogs, when applied to their nasal mucous membrane, the clinical symptoms of distemper.

Torrey and Rahe, like McGowan and Ferry, found that the subcutaneous and intraperitoneal inoculation of dogs did not give rise to typical distemper symptoms, they therefore, believing that the respiratory tract under normal conditions is the portal of entry for infection, tried the effects of blowing into the mouth and nose of dogs mixtures of sterile dust and *B. bronchisepticus*. They state that after an incubation period of 3 to 13 days, definite symptoms of the disease developed in 7 out of 8 of these animals, the attacks ranging as is the case in natural distemper, from very mild to very severe and fatal issues. The symptoms developed included rhinitis and bronchitis with persistent cough, typical bronchopneumonia, vomiting, bloody diarrhœa, conjunctivitis, and in three instances the appearance of a typical pustular eruption on the skin. Similar results were obtained by spraying emulsions of *B. bronchisepticus* into the nose and mouth of the dogs and by intratracheal inoculation.

Immunization.

Torrey and Rahe found that recovery from an attack of experimental distemper induced by injection with *B. bronchisepticus* seemed to confer a sure immunity to natural distemper. They found that the dogs which had developed catarrhal symptoms of distemper after the introduction of the bacillus into the respiratory tract, and had completely recovered remained well during a long exposure to cases of natural distemper of all degrees of severity. They also state that susceptible dogs may be protected against distemper by the subcutaneous injection of a vaccine prepared with *B. bronchisepticus*.

Diagnosis and Results of Infection.

Ferry in an epidemic of distemper was able to cultivate *B. bronchisepticus* from the trachea or from the entire respiratory tract of animals that were killed early in the disease, and frequently in pure culture. Later in the disease the presence of other bacteria renders detection and isolation more difficult. Ferry, but not McGowan, found the agglutination test useful in diagnosis. Torrey and Rahe found agglutinins invariably present in dogs suffering from distemper and absent from normal dogs which had not passed through an attack or been exposed to the disease. They concluded that the test was of little value in diagnosing the disease, unless there is a definite history of no previous attack and no exposure to infection. McGowan realized, however, the difficulties of excluding from his experimental animals infection from natural sources; so that he is very guarded in the inferences that he drew from his careful experiments. He believed that distemper was not a disease confined to the dog, but that the cat and various laboratory animals were liable to it, and that the clinical condition was of a very protean type. He noted

the presence of *B. bronchisepticus* in the nasal mucous membrane of apparently healthy dogs, cats, rabbits, and guinea-pigs, and regarded such as healthy ' carriers '.

Pathological lesions. In animals which had suffered from natural distemper, McGowan found that the lesions were confined to the eyes, nose, trachea and lungs. The eye conditions were conjunctivitis, chemosis, ulceration of cornea, interstitial keratitis. In the nose there was seropurulent inflammation frequently extending into the adjoining sinuses. In the trachea there was tracheitis, whilst the lungs resembled those in cases of inhalation pneumonia—patches of consolidation—collapse with exudation of pus from the bronchi and bronchioles when a section is made across an affected lung.

Ætiological Significance in Distemper.

Immunization. If it were shown that vaccination with *B. bronchisepticus* prevented infection of susceptible animals when exposed to contact with animals suffering from the natural disease, then the claim of *B. bronchisepticus* to an ætiological relationship with the disease would be strengthened. McGowan (1911) and Ferry (1911) carried out an experiment of this nature with apparent success.

Hardenbergh (1926) and Pugh (1926), however, state that the Research Committee of the British *Field* Distemper Council have frequently failed to isolate *B. bronchisepticus* from cases of canine distemper, and that this micro-organism cannot seriously be considered as the primary infective agent, which is probably as Carré believed a filterable virus. It would seem that *B. bronchisepticus* is related to canine distemper in much the same way as *B. suipestifer* is related to hog cholera—a fairly frequent secondary invader. The recent studies of Dunkin and Laidlaw (1926) indicate that the infecting agent of dog-distemper belongs to the class of filter-passing viruses, and rule out *B. bronchisepticus* as the causative factor (see Vol. VII).

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The Proteus Group.

The Group as a Whole.

Definition and Description.

The proteus group includes those species which occur as rods varying from short coccoid forms to filaments; which are Gram-negative, non-spore-forming, generally actively motile and possessing peritrichous flagella; which are aerobes and facultative anaerobes; which liquefy gelatin, and often in this medium produce 'swarm colonies' with radiating outgrowth; which often form rapidly spreading growths on agar; which under aerobic conditions decompose proteins; which utilize amino-acids and carbohydrates; and which may be saprophytic or parasitic in nature. They cause coagulation and digestion in milk, and in broth there is a uniform turbidity with a later formation of a thin pellicle. They may or may not form indole, and the type species does not produce acetyl-methyl-carbinol.

Members of the group. B. proteus vulgaris *and* B. proteus mirabilis.

The Relationship of the Group and of its Members.

The term Proteus first used in bacteriological nomenclature by Hauser, (1885), signifies changeability of form as personified in the Homeric poems by Proteus 'the old man of the sea', who had the gift of endless transformation. The bacilli are themselves very pleomorphic, and the colonies in 5 per cent. gelatin often consist of 'swarming islands' connected by strands of growth. Hauser described three types of bacilli which he isolated from putrefying fluids, and which he called *Proteus vulgaris, P. mirabilis* and *P. zenkeri.* The latter differed from the two others in not possessing gelatin-liquefying powers, whilst of the two others, *Proteus vulgaris* was the more actively proteolytic. Later, Hauser regarded *P. mirabilis* and *P. zenkeri* as attenuated forms of *P. vulgaris.* At present

most writers have recommended the removal of *P. zenkeri* from the *Proteus* genus. It differs in being Gram-positive, and in being unable to ferment carbohydrates and to digest proteins and gelatin. Before Hauser's work appeared, Kurth (1883) had isolated from the intestine of fowls the *B. zopfii* with which Wenner and Rettger (1919) state that *B. zenkeri* is identical. Bergey (1926) has created a new genus *Kurthia* in which these two species—*Kurthia zopfii* and *Kurthia zenkeri* appear.

Wenner and Rettger (1919) suggested the employment of maltose as a means of classifying members of the Proteus group; the maltose fermenters and non-fermenters being put into *Proteus vulgaris* and *P. mirabilis* subgroups respectively. This suggestion has been adopted by Bergey (1926), who employs their action on mannitol, maltose and gelatin as a means of differentiating various species. These he divides into two groups; (*a*) those forming no acid or gas in mannitol; (*b*) those fermenting mannitol. The former contains the majority of the Proteus group, and its members are subdivided into two species *Proteus vulgaris* and *Proteus mirabilis* according to their action on maltose. Of the mannitol fermenters Bergey describes three species, *Proteus asiaticus*, *Proteus valeriæ* and *Proteus hydrophilus*, the first two being unable to liquefy gelatin, whilst *P. hydrophilus* liquefies gelatin and also is able to ferment lactose with the formation of acid without gas.

P. asiaticus is described as non-motile and inactive on salicin, whilst *P. valeriæ* is motile and forms acid and gas in salicin media. Whether these three species should be included in the Proteus group at all will depend on how the group is defined. The most characteristic biochemical and cultural properties of the group are: (*a*) the liquefaction of gelatin and the digestion of proteins; (*b*) a spreading growth on agar; (*c*) the possession of a powerful urea-splitting ferment. Although on prolonged cultivation most of these properties may be lost, and when this occurs, it is a matter of difficulty in deciding whether an organism should be classed in the *B. coli* or in the *B. proteus* group, yet there is no evidence that *B. valeriæ* and *B. asiaticus* ever liquefied gelatin or serum, fermented urea, or grew in a spreading film on agar. Moreover, their discoverers, Boycott (1906) and Castellani (1912), regarded them as being closely related to the *B. paratyphosus* B group.

Mannitol as a rule is not fermented by *B. proteus*, but occasionally organisms in other respects identical with *B. proteus* possess this property, which, however, they frequently lose in the course of cultivation. *Proteus hydrophilus* differs from such strains in that it forms acid but no gas in lactose. The Proteus group must be regarded as a non-lactose-fermenter in the laboratory sense, although Wolf (1919) has shown that in milk cultures, lactose disappears to the extent of 16 per cent. in 144 hours.

The *Bacillus cloacæ* of Jordan is intermediate in position between the *B. coli* and *B. proteus* groups. It liquefies gelatin and ferments lactose, though sometimes the action is slow, usually ferments mannitol, and invariably gives a positive Voges-Proskauer reaction.

The spreading growth of *B. proteus vulgaris* may be lost and colonies having the appearance of those of *B. coli* may develop and such continue to breed true. Weil and Felix (1917) noted in old cultures of 'X 19' strains of *B. proteus*, two types of colonies (1) the 'H' (Hauch) type with characteristic filmy formation, and (2) the 'O' (ohne) with well-defined margin and raised surface. A strain with the 'O' character of growth, although belonging to the Proteus group, shows affinities with that of *B. coli*. These strains sometimes lose (Schaeffer, 1919) their power of liquefying gelatin and of digesting protein, and if they happened to possess the capacity of fermenting mannitol, without a knowledge of their previous history, it would be difficult to differentiate them from *B. coli*. The group shows certain affinities with that of *B. pyocyaneus* and *B. fluorescens*, but is distinguished by inability to form the corresponding pigments. The only pigment formed by the Proteus group is a brown one, seen best in growth on potato, and also to a less extent in bouillon.

Earlier workers regarded *B. proteus* as the prototype of a putrefying microbe. From the results of the work of Rettger and Newell (1912–13), Wenner and Rettger (1919) and Wolf (1919) this position can no longer be held. Of course, much depends on the definition of putrefaction, and that suggested by Rettger and Newell (1912–13) would be acceptable to most —'a particular process of protein decomposition which is brought about through the agency of bacteria, with the evolution of foul-smelling products which are characteristic of cadaveric decomposition'. Such decomposition is mainly found under anaerobic conditions, and here, no doubt, *B. proteus* by its growth and absorption of oxygen facilitates the work of the obligatory putrefactive anaerobes, e.g., *B. sporogenes*, but it has been found that in the absence of oxygen *B. proteus* is unable to liquefy gelatin or digest protein. Oxygen is essential for the production of the proteolytic enzyme. Though *B. proteus* is apparently often saprophytic in its nature, it has shown itself to be parasitic on numerous occasions. It does not appear to be present in large numbers in the healthy human intestine, but is frequently associated with *B. typhosus*, *B. dysenteriæ*, &c., and it is thought by some that it may aggravate the disease in dysentery. The part played by *B. proteus* in infantile gastro-enteritis and diarrhœa is discussed in another section (p. 317). It is rare that *B. proteus* is the causative organism in outbreaks of food-poisoning.

As regards pathogenicity to mice, Bengtson (1919) found that 1 c.cm. of a broth culture, regardless of its source, caused death and that 0·1 c.cm. produced no ill-effects.

B. PROTEUS VULGARIS.

This organism may be described as a Gram-negative motile rod with peritrichous flagella, non-spore-forming and non-capsulated, aerobic and facultatively anaerobic, usually with a spreading growth on agar and capable of liquefying gelatin, and digesting protein and frequently of forming indole, capable of precipitating and dissolving casein in milk,

and of fermenting with the production of acid and gas glucose, galactose, saccharose and maltose, but having no action on lactose and mannitol. It was isolated by Hauser in 1885 from decomposing animal matter, and this is where the organism is most frequently encountered. It is mainly saprophytic, but either alone or more frequently in association with other bacteria, especially staphylococci and streptococci, it can infect the animal body.

Morphology.

Cultures grown on agar for 24 hours at 37° C. show, as a rule, rods varying from 0·8 to 3μ in length and from 0·3 to 0·5μ in breadth. Variation in size is common, and is a point on which Hauser laid considerable stress. In certain cultures, gradations from coccoid forms to bacilli 5 to 8μ long are encountered, and in certain strains long chains of streptobacilli are seen. Spiral forms and long filaments may be seen, but the occurrence of branching is not described. The bacillus stains readily with the ordinary basic coal-tar dyes, and is Gram-negative.

Cultivation.

Temperature. Levy (1894) states that growth develops slowly at a temperature as low as 0° C. and as high as 43 to 45° C. Wenner and Rettger (1919) invariably obtained maximum growth at 34 to 37° C.

Reaction. A pH of 6·0 to 7·0 (Dernby, 1921) is the most suitable for the growth of the bacillus, but it readily adapts itself to media more alkaline than this. The hydrogen ion concentrations at which growth occurs vary from pH 4·4 to 8·4 (Dernby). Growth in plain broth is accompanied by slight alkali-production, and in the same medium containing a fermentable carbohydrate acid is produced, bringing the hydrogen ion concentration to about pH 5 on the colorimetric scale.

Oxygen. Although the organism can develop either under aerobic or anaerobic conditions, the presence of oxygen is essential for the production of the proteolytic enzyme (Wenner and Rettger, 1919).

Chromogenesis. The only colour production described is the development of a slight brownish colour in broth (Bengston, 1919) and the browning of potato. Fejgin (1924) describes variant forms which produce a yellow pigment.

Smell. A putrefactive odour is produced and sulphuretted hydrogen is developed in peptone media. Under anaerobic conditions no putrefaction occurs. Mercaptan was tested for by Wenner and Rettger but with negative results. The bacillus is unable to reduce sulphites to sulphides in the presence of a fermentable carbohydrate (Wilson and Blair, 1927).

Dyes. The dyes employed in connection with the isolation of *B. typhosus* on special media have no inhibitory action in these concentrations on the growth of *B. proteus*. For instance, it grows well on media containing crystal violet, malachite green and brilliant green.

Growth in bouillon. Turbidity is rapidly produced, reaching a maximum in 3 to 5 days at 30 to 37° C. Older cultures develop a thin brittle pellicle. Nitrates are reduced to nitrites.

Growth on plain agar. A spreading film of growth rapidly develops on slant agar or on a moist agar plate. Choukévitch (1911) made use of this property for the isolation of *B. proteus* from a mixture. His method consisted in adding the material to be investigated to gelatin and inoculating the condensation fluid of an agar slant with growth obtained from liquefied areas of the gelatin. *B. proteus* spread upwards over the agar surface, and at the top was found in practically pure culture. On drier agar rosette-like colonies with irregular borders are formed. Colonies, small, raised, and with entire margin, resembling those of *B. coli* are sometimes found, these are the ' O ' colonies of Weil and Felix. A culture of *B. proteus* may contain a mixture of organisms forming these two types of colony : (1) the ' H ' or spreading filmy growth ; (2) the raised colony with well-defined edge. The ' O ' type was obtained pure by plating old broth cultures of the ordinary or 'H' form on agar. Later observations by Braun (1918) and by Braun and Schaeffer (1919) showed that in cultures on carbolic agar or in media deficient in nutriment, the colonies were devoid of filmy outgrowths and the bacteria were essentially of the 'O' type, but reversion took place to the 'H' form when subculture was made on ordinary nutrient agar (Felix and Mitzenmacher, 1918). The ' O ' type of colony consists of non-motile bacilli unprovided with flagella (Braun and Schaeffer, 1919) or with only a single flagellum (Miller, 1927). Its antigen is very resistant to heat, whereas this is only true of part of the antigen of the ' H ' type. This is an important matter in connection with the Weil-Felix reaction.

Growth on gelatin. The liquefaction of gelatin rapidly follows the development of irregular spreading growth on a plate ; in a tube stratiform liquefaction occurs and soon the whole of its contents are rendered fluid. Strains under cultivation may lose their power of liquefying gelatin or digesting protein ; one or other or both of these capacities may be lost (Schaeffer, 1919) and may be restored by passage through a mouse (Herter and TenBroeck, 1911). The non-liquefying strains remain typical in other respects, and in no way resemble *B. zenkeri* or *B. zopfii*.

Selective media. By the addition of carbolic acid to agar, the spreading growth is prevented, and it is thus easier to obtain isolated pure colonies. A medium (Wilson and Blair, 1927), which inhibits the growth of *B. coli* but allows that of *B. proteus*, has greatly facilitated the isolation of *B. proteus* from fæces, milk, &c. It contains liquor bismuthi, sodium sulphite and brilliant green.

Growth in milk. Rapid development occurs, and if litmus is present the colour is bleached. Litmus cannot, therefore, be employed to determine the changes in reaction, and its use may account for the different results obtained. Bengtson (1919) used brom-cresol-purple as an indicator, and found that the majority of *B. proteus* cultures were slightly acid in 24 hours,

but showed no curdling until after a lapse of 3 days, when curdling and the beginning of peptonization were apparent. Wenner and Rettger (1919), in a study of 73 strains, found 3 showing slight acid production at first; in the other 70 the changes were an initial alkalinity, which became more intense and was followed by decolorization of the litmus and digestion of the casein.

Bacteriophage.

Fejgin (1924) claims to have brought about modified strains of *B. proteus*, strain X19, by acting on cultures with a bacteriophage. The four variants showed marked differences from the parent germ. Three produced a yellow pigment, were inactive on sugars, failed to produce indole and liquefied gelatin very slowly. The results await confirmation.

Biochemical Reactions.

Action on Proteins.

Peptonization. Mainly through the work of Rettger and his associates, it has been shown that the decomposition of native albumens and peptones by *B. proteus* is not due to the direct action of the bacillus itself, but to a proteolytic enzyme elaborated by it, and that for this latter purpose oxygen is necessary. The presence of fermentable carbohydrates in the media inhibits the formation of indole—an index of proteolytic action—and also the liquefaction of gelatin. This appears to be due to the interference with the production of the enzyme, for the presence of sugars does not inhibit the action of filtrates containing the tryptic enzyme. The curdling of milk is due to an enzyme, and not to acid production.

Putrefaction. As already mentioned the action of *B. proteus* differs from that of the active agents of putrefaction—the spore-forming anaerobes—in that no decomposition occurs in the absence of oxygen.

Indole. There is great variation in the production of indole by different strains of *B. proteus*. Probably more strains are inactive than are active in this respect. Steensma (1906) described several strains which failed to produce indole, and of 61, 24, 29 and 73 strains examined by Berthelot (1914), Horowitz (1916), Stewart (1917), and Wenner and Rettger (1919), the indole formers numbered 24, 7, 1 and 69 respectively. Bengtson's (1919) results supported the view of Cantu (1911), that the production of indole is more marked in cultures derived from animal sources, and she called attention to Horowitz's comment that strains which gave a positive indole test as a rule fermented saccharose. Van Loghem and Van Loghem-Pouw (1912) made two subdivisions of *B. proteus* strains on the basis of their indole-producing capacity—*B. proteus indologenes*, and *B. proteus anindologenes*. Most authorities, however, would agree with Wenner and Rettger in regarding indole production as unsatisfactory as a distinguishing character.

Action on carbohydrates.

Wenner and Rettger (1919) conclude that fermentation is limited to glucose, lævulose, galactose, saccharose, maltose and glycerin, which are

broken up with the production of acid and gas, whilst no action was shown on lactose, inulin, dulcitol, mannitol, sorbitol, salicin, raffinose, arabinose, adonitol, dextrin and starch. The same writers noted a variation in the action of *B. proteus* on saccharose, some strains producing the maximum amounts of acid and gas in 24 to 74 hours, whilst others required 12 to 15 days. Of the 73 strains studied, 25 showed an immediate and 48 a delayed action. It was observed that the strains which fermented saccharose readily also fermented maltose whilst the others did not. The action of a strain on maltose is adopted by Bergey (1926) in subdividing the *B. proteus* group. Strains fermenting or not fermenting this sugar are classified as *B. proteus vulgaris* and *B. proteus mirabilis* respectively.

Bengtson's (1919) results are similar to those reported above, but in her series she describes a few strains which differed from the others in their fermentation reactions, although in other respects belonging to the *B. proteus* genus ; for instance, culture 63 (meat) fermented dextrose, saccharose, maltose, mannitol and raffinose ; culture 69 (blood) fermented maltose and mannitol, but failed to ferment saccharose, and culture 49 produced no gas but acid in dextrose, saccharose, maltose, and mannitol. This culture, however, produced gas in dextrose and saccharose broth in certain preliminary tests. No doubt there is considerable variation in fermentation results, and whether these variations would warrant the creation of new species is doubtful. It is probable that ability to ferment mannitol should not exclude a strain from the *B. proteus vulgaris* species, although this power is more frequently absent than present. Horowitz (1916) in a study of cultures isolated in an epidemic of gastro-enteritis, found glucose fermented by all strains with acid and gas production, and maltose and mannitol fermented by all except one. Bengtson (1919) after a survey of the question concludes : ' It seems probable that fæcal strains often ferment maltose, saccharose and mannite when freshly isolated, and later lose the power to a greater or less extent, and sometimes completely '.

Bengtson and others have found that fermentation of a sugar may occur in bouillon and not in peptone water, and suggest that the meat extract may contain substances (hormones) which increase the metabolic activities of certain strains as regards carbohydrates. Rochaix and Sarda (1926) consider that in their ability to ferment æsculin and salicin, and in their failure to ferment lævulose ' X19 ' strains differ from those of ordinary *Proteus*. Wilson (1927) found that all the strains of X19, with the exception of ' Kingsbury ', fermented salicin and that other *Proteus* strains were inactive. As regards lævulose, he found that many other strains of *Proteus* besides X19 have no apparent action on this carbohydrate.

Voges-Proskauer Reaction, Reductions, Oxidations, Hæmolysis.

There is no record of a typical *B. proteus* strain producing acetyl-methyl-carbinol, and giving a positive V.-P. reaction. This is an important point of distinction between *B. proteus* and *B. lactis aerogenes* and *B. cloacæ*.

Reductions and oxidations. Nitrates are reduced to nitrites and ammonia. The production of sulphuretted hydrogen in peptone water is a constant phenomenon. Litmus methylene blue and indigo are reduced to colourless compounds. Jacoby (1919) found a much smaller catalase content in X strains than in the ordinary strains of *Proteus*. No reduction of sulphite occurs in the presence of a fermentable carbohydrate—an important distinction between *B. proteus* and *B. typhosus* and *B. paratyphosus* B and other members of the Salmonella group (Wilson, 1923).

Hæmolysis. Bach (1921) and Braun and Shi-Tsing (1922) have shown that a hæmotoxin is present in young broth cultures of *B. proteus*. Maximum hæmolysis of sheep and rabbit cells occurs with cultures 5 to 6 hours old, then the power gradually falls and after 24 hours may have disappeared. The hæmotoxin is not found in filtrates and is diminished and destroyed by exposure to 65° C. for 30 minutes. It seems not to be a true hæmotoxin as it is impossible to prepare an antihæmotoxin. Weinberg and Otelesco (1921), in a study of eight strains, found that all produced a hæmolysin.

Serological Reactions.

Klieneberger in 1908, reviewed the literature bearing on the question of the unity or heterogeneity of *B. proteus*. His own experiments with immune sera obtained from rabbits inoculated with four strains derived from infected human tissue showed that not only was the homologous strain agglutinated, but also several others. He considered that *Proteus* strains formed a biological entity in the matter of agglutination just like *B. typhosus*. Horowitz (1916) tested a number of strains from diarrhœic stools, and found that they fell into five serological groups. Bengtson (1919) prepared five immune sera and found that the *B. proteus vulgaris* sera agglutinated many other strains besides the homologous, but there was no clear cut division into types. Wenner and Rettger (1919) prepared seven immune sera from as many strains of *B. proteus*, and found that with one exception all the sera agglutinated strains other than those used in their preparation. Nineteen strains were not agglutinated by any of the seven immune sera. Of 73 strains examined, 10 were agglutinated by serum A, 13 by B, 25 by C, 42 by D, 11 by E, 25 by F, and 1 by G. They concluded that the *Proteus* group is more or less heterogeneous. Aoki and Iizuka (1920) by cross-agglutination tests with the sera of rabbits immunized with 34 strains (20 being *P. vulgaris* and 14 *P. mirabilis* according to Hauser's classification) found that the 20 strains of *P. vulgaris* fell into 7 subgroups, and the 14 of *P. mirabilis* into 5 subgroups.

All the workers agree that fermentation reactions did not by any means always correlate with the agglutination reactions in the tests. These attempts at classification by agglutination are to some extent vitiated by the fact that the possibility of different reactions of the heat-labile and stable antigens were not taken into account.

Braun and Salomon (1918) examined critically the serology of a number of 'X' strains and compared them with other strains of *B. proteus*.

They did not endorse the views of Weil and Felix as to the specificity of the reaction although they considered the antigenic constituents separately. Weil and Felix (1921), Zlocisti (1920) and Wolff (1922) have further dealt with the whole subject.

Fletcher and Lesslar (1925, 1926) found that an immune rabbit serum prepared with the anindologenic strain ' Kingsbury ', which unlike other X19 strains had no action on saccharose or maltose, agglutinated these strains to almost full titre and that absorption with any of them removed all the agglutinins from the serum. They found, however, that sera homologous for the other strains agglutinated the ' Kingsbury ' strain only to 12 and 25 per cent. of the full titre, and that absorption with ' Kingsbury ' did not remove these agglutinins. In the section on *Proteus* 'X19' (p. 318), further reference is made to the serological grouping of *Proteus* strains.

Pathogenic Action.

In man. B. proteus is frequently found in association with other organisms in various inflammatory conditions in man, e.g. otitis media, cystitis, gastro-enteritis, peritonitis, war-wounds and gas gangrene. It is only rarely that the bacillus is found alone or as the primary infecting agent. Outbreaks of food-poisoning have been attributed to the presence of this organism and its products in foodstuffs. The evidence on which this view was based was, in the majority of instances, inconclusive. As is well known, Metchnikoff and his associates believed that it played a primary role in the causation of infantile diarrhœa. Rarely is it the cause of a septicæmia, and seldom has it been obtained in blood cultures : but instances of this are reported by Maymone (1917), Warren and Lamb (1924), and Irimonoiu and Popa (1925).

In animals. Mice, rats, guinea-pigs and rabbits can be killed by subcutaneous or intraperitoneal injection of 0·5 c.cm. of broth cultures, especially with strains isolated from infantile diarrhœa (Tsiklinsky, 1917). Frequently death results within 24 to 36 hours ; where the animals survive abscesses are often formed and wasting is observed. Strains freshly isolated from pathological sources may produce definite lesions, e.g. abscesses, enlargement of spleen, hæmorrhage of the intestine and diarrhœa. More frequently no definite lesions are present and death occurs from toxæmia. Bengtson (1919) carried out virulence tests with strains from various sources, injecting mice with 1 c.cm. and 0·1 c.cm. amounts of broth cultures. In general, she found that 1 c.cm. of broth cultures injected subcutaneously caused death of mice within 24 hours, and that 0·1 c.cm. had no effect, no matter what was the source.

Endotoxin. Cultures on agar and in broth are equally toxic. No evidence has been advanced in favour of an extracellular toxin for which an antitoxin could be prepared. Bengtson (1919) refers to the work of Levy, Fornet and Heubner, Meyerhof, Herter and TenBroeck, and Berthelot, in their attempts to isolate a toxin. Levy, by treating a liquefied gelatin culture of *B. proteus* with absolute alcohol and precipitating

with calcium chloride and drying, obtained the toxic substance ' Sepsin ', which produced the same effect as living cultures, killing dogs injected intravenously with 1 gm. of the sterile powder and rabbits injected intravenously, intraperitoneally or subcutaneously with 0·2 to 0·3 gm. Symptoms of vomiting, bloody diarrhœa and rise of temperature were present and hæmorrhagic infiltration of the intestine and enlargement of the spleen were found on necropsy. The results obtained by the above workers in Bengtson's opinion indicates that *B. proteus* produces a very weak soluble toxin as compared with that of *B. tetani* or *C. diphtheriæ*.

The effects of ingestion of the bacilli. Bengtson reviews the evidence in favour of an ætiological role of *B. proteus* in outbreaks of food-poisoning and finds it unconvincing. The writer has investigated an outbreak of diarrhœa in an Officers' Mess which, he believed, was due to *B. proteus* present in large numbers in the soup and in the evacuations of the patients. Nine were attacked and three who had not taken any soup at dinner escaped. It is noteworthy that it is only the older outbreaks of food-poisoning which were attributed to *B. proteus ;* in recent years almost invariably a member of the Salmonella group has been incriminated.

Metchnikoff was able to cause the death of chimpanzees and of young rabbits by adding to their food pure cultures of *B. proteus* isolated from cases of infantile diarrhœa. It is probable that *B. proteus* and the products of its growth in milk are of importance in the causation of the diarrhœa of infants, but further work is required before the point can be decided. Demnitz (1926), in a patient suffering from diarrhœa accompanied by fever, isolated the same strain of *B. proteus* from his stools and from a blood sausage of which he had partaken. The patient's serum agglutinated this strain in 1 in 400 dilution, and was without action on *B. typhosus* and on *B. paratyphosus* A and B.

Viability.

The remarks that have been made in reference to the *B. coli* group apply to that of *B. proteus*. *B. proteus* is more tolerant of certain coal-tar dyes than is *B. coli ;* for instance, brilliant green and malachite green, which in a certain concentration inhibit the growth of many strains of *B. coli*, have no such effect on that of *B. proteus*. *B. proteus* is also tolerant of the action of bismuth and brilliant green in sulphite media, and this fact has been utilized for its isolation (Wilson and Blair, 1927).

Distribution.

B. proteus is widely distributed, but it is by no means ubiquitous. The numbers found in soil, water and on grains are far fewer than those of *B. coli*. Its chief habitat is in decomposing animal matter. In meat products, e.g. sausages in which no noticeable decomposition has occurred, Cantu (1911) has demonstrated the organism in one-third of the samples examined. Burton and Rettger (1917) rarely met with it in soil and concluded that its habitat was elsewhere, but Cantu has isolated it from 44 per cent. of the samples of soil examined by him.

In animals and man: Occasionally *B. proteus* has been found in the mouth secretions and on the surface of the human body. In normal stools, *B. proteus* is either absent or present in only scanty numbers (Feltz, 1899; Cantu, 1911; Stewart, 1917; Bengtson, 1919). In enteric fever (Wilson and Blair, 1927), and in dysentery *B. proteus* is often found. In the stools of normal infants *B. proteus* is not of frequent occurrence; thus Bertrand (1914) obtained positive results in 2 out of 24 specimens, Bahr (1912) in 2 out of 27, whilst Horowitz's (1916) results were negative in 40 examinations. On the other hand, Metchnikoff (1914) found in the stools of infants suffering from gastro-enteritis *B. proteus* in 96 per cent. of his examinations. Bertrand (1914) was able to demonstrate *B. proteus* in the stools of 55 infants suffering from diarrhœa in London, and Tsiklinsky (1917), in 65 per cent. of the cases studied by him, chiefly in Moscow. This author also isolated *B. proteus* from the stools of 20 per cent. of normal infants, but found the cultures not so virulent for rabbits as those isolated from cases of gastro-enteritis.

Natural resistance of man and animals. In the adult healthy intestine *B. proteus* is unable to establish itself in the manner of *B. coli*. Probably the hydrochloric acid of the gastric juice destroys the majority of those reaching the stomach. From the frequent occurrence of *B. proteus* in inflamed surfaces, e.g. enteric or dysenteric ulcers, war wounds, &c., it would seem that the secretions offer a suitable pabulum for the development of the germ.

Reaction to infection. Normal serum has a low content of agglutinins for *B. proteus*, probably below 1 in 40 dilution. In certain definite cases of infection with *B. proteus*, e.g. septicæmia, suppurative infections, &c., the titre has been found as high as 1 in 6,000. In infantile diarrhœa, the *Proteus* strains isolated from the bowel are rarely agglutinated in a dilution of 1 in 20 (Horowitz, 1916).

Acquired resistance. Vaccines have been of service in my experience in the treatment of cystitis, endometritis, &c., associated with *B. proteus*.

B. PROTEUS MIRABILIS.

This species is closely allied to *B. proteus vulgaris*, from which it differs in not forming acid and gas in maltose and in not forming indole. In other respects it conforms to the description of *B. proteus vulgaris*. Whether it is wise to classify it as a separate species is doubtful. We have clearly seen that classification by agglutination and fermentation tests lead to discordant results. However, for convenience it is an advantage to group together organisms which have many points of resemblance.

B. PROTEUS 'X19'.

Considerable interest in the *B. proteus* group was aroused by the work of Weil and Felix (1916, 1917, 1921) on the agglutination by the sera of typhus-fever patients of certain strains designated 'X2' and 'X19', which had been isolated from the urine or blood of a few cases. Prior

to this, Wilson (1909 and 1910) had shown that the sera of typhus-fever patients agglutinated non-lactose-fermenters and coliform bacilli present in the urine of such patients. An extensive literature has grown up regarding the phenomenon and reviews of it are given by Rocha-Lima (1919), Zlocisti (1920), Wolff (1922), Schiff (1924) and by Wilson (1920 and 1927). The original ' X2 ' and ' X19 ' strains in their cultural and agglutinative characters corresponded to those of *B. proteus vulgaris*. Recently it has been found by Rochaix and Sarda (1926) and Wilson (1927) that they differ from most strains in that they ferment salicin.

Fletcher and Lesslar (1925 and 1926) and Wilson (1927) find that the X19 strains with one exception are identical in cultural characters. The exception is a strain designated ' Kingsbury ' which Fletcher and Lesslar found did not ferment maltose or saccharose, did not form indole, and was agglutinated by the sera of certain cases of tropical typhus which had no action on the other strains. Wilson (1927) found that the strain ' Kingsbury ' did not ferment salicin.

Fletcher and Lesslar (1925 and 1926) found that the sera in cases of tropical typhus occurring in the Federated Malay States were of two kinds : one agglutinating ordinary X.19 strains which belong to the *indologenes* or *B. proteus vulgaris* species, and the second with little or no effect on these strains, but agglutinating ' Kingsbury ', which so far as could be ascertained was descended from a culture of X.19 obtained from the British National Collection of Type Cultures in 1921.

Wilson-Weil-Felix Reaction.

The cardinal fact underlying the serological diagnosis of typhus fever is the development of agglutinins in the blood of the patients. The bacilli most sensitive for the detection of these agglutinins are members of the *Proteus* group, e.g. X2, X19, X19 strain ' Kingsbury ', XV (Silber, 1927), but other organisms, e.g. *B. pyocyaneus* (Kreuscher, 1918 ; Neukirsch and Kreuscher, 1919 ; Sampietro, 1920 ; Wilson, 1922), *B. agglutinabilis U2* (Wilson, 1927), are often found almost equally serviceable. The technique of the reaction is described elsewhere (Vol. IX). In the view of Weil and Felix the agglutination of Proteus X19 by the blood of typhus patients is essentially ' specific ' and due to a special relation between the virus of this disease and this particular bacillus (Weil and Felix, 1918). On the other hand, the present writer, on the ground of his own experimental work with *Bacillus U*, U_2, &c., and as the result of review of the work of others who have tested typhus serum with other kinds of bacteria, considers that Proteus X19 is only one, though the most suitable, of a number of organisms which can be used for diagnostic agglutination in typhus fever. According to the latter view, the reaction is due to the production of agglutinins by the typhus patient for a variety of bacteria and is not really specific (Wilson, 1909, 1910, 1917, 1920, 1923, 1927).

Some of the arguments against the specific action of X strains in typhus fever are : (1) the heat-lability of the agglutinins found in the serum of

typhus-fever patients compared with the greater heat-stability of the agglutinins in the serum of a man or animal inoculated with cultures of the X strains (Csépai, 1917; Hamburgher and Bauch, 1917; Fairley, 1919); (2) differences are displayed with regard to the action of typhus fever agglutinins and specific agglutinins in the serum of immunized rabbits on heated and unheated bacilli (Dietrich, 1916; Sachs, 1917; Sachs and Schlossberger, 1919; Aoki and Hashimoto, 1922); (3) the rarity of the isolation of X strains from the bodies of typhus-fever patients (Felix, 1917; Schürer and Wolff, 1919); (4) the occurrence of X strains in the bodies of people who have never had typhus fever and who have never been in contact with such cases. This point appears to the writer to be most important and can be said to be definitely established from the observations of Dienes (1917), Much and Soucek (1917), Finger and Kollert (1918), Weltmann and Molitor (1919), Wolff (1922), Aoki and Kondo (1922), Kollert and Bauer (1925), and Wilson (1927). Many, e.g. Papamarku (1918), Öettinger (1918), Grütz (1919), Silber (1923), regard the agglutinins as paragglutinins, and among others Silber (1927) has shown that an ordinary *proteus vulgaris* strain kept in a collodion sac in the abdominal cavity of a guinea-pig infected with typhus becomes agglutinable by typhus-fever serum. The strain that Silber employed was called XV and was found by him after the above treatment to remain agglutinable even after five years' cultivation. He showed that absorption of a typhus-fever serum with XV left the agglutinins for X19 and X2 intact and that absorption with X19 did not influence the XV agglutinins. It was already well known that absorption tests had shown that the agglutinins in typhus serum for X2 and X19 were separate and distinct (Braun and Salomon, 1919). The finding of agglutinable X19 strains under natural conditions renders the paragglutination theory unnecessary; moreover, this theory affords little or no explanation of what is the crux of the problem—the steady rise and decline of heterologous agglutinins in the typhus-fever serum.

It would seem that all we know at present is that agglutinins for numerous bacteria, but especially for certain *Proteus* strains, are developed in the course of typhus fever and that these may result from the possession by the typhus virus of an antigen common to itself and to certain bacteria, or from the development of certain and constant physical and chemical alteration of the serum resulting directly or indirectly from the response of the body to infection with the virus.

Inoculation of rabbits with the brain of typhus-infected guinea-pigs causes the development of agglutinins for X19 in their sera, but is without result if the material has been previously heated to 58° C. for half an hour (Weil and Felix, 1920). We might regard the condition as being due to an increase of normal agglutinins (Paneth, 1917; Schaeffer, 1919), but we should still be not much enlightened, as no satisfactory explanation of how normal agglutinins are produced is yet available.

' O ' and ' H ' Strains.

Weil and Felix (1917) sought to meet the objections raised as to the specificity of their X strains by demonstrating that cultures of X19 dissociated into two varieties, one forming a raised colony without any spreading film (ohne hauch)—' O ' strains, and the other possessing this outgrowth—' H ' strains, and by showing that the ' O ' variety was agglutinated in highest titre by typhus-fever sera, and that the agglutinins produced in the blood of rabbits inoculated with ' O ' strains behaved as to heat-lability like the agglutinins in typhus-fever serum. Whatever may prove to be the relationship of *Proteus* strains to typhus fever, the work of Weil and Felix (1917) on the splitting of their X19 culture into two types is of immense importance in connection with the study of bacterial variation and of the serological grouping of bacteria. Weil and Felix (1917) divided *Proteus* bacilli into three groups according to the agglutinating action on them of sera prepared by inoculating rabbits with either X2 or X19 cultures. Bacilli in Group I were not agglutinated, bacilli in Group II were agglutinated to about 1/5 of the titre, and bacilli in Group III were agglutinated to full titre. Group III included not only the specific X strains, but also certain saprophytic strains, and on these latter the sera of typhus-fever patients had no action. Weil and Felix explained this difference by showing that the *Proteus* strains possessed two receptors which they designated ' spezifisch ' and ' substanz ' (entire) receptors and that the latter was common to the X strains and the Group III strains, whereas the ' specific ' receptor was peculiar to the X strains and different from that possessed by the other *Proteus* strains. These two receptors have been in subsequent papers called the heat-stable or O and the H receptors respectively. And it is recognized that the H receptors consist of two parts, one identical with the O and the other heat-labile and only found in the H form. The two receptors or antigens are also recognized as analogous to the ' somatic ' and ' flagellar ' receptors of Th. Smith and Reagh (1903) and occur in *B. typhosus*, Salmonella, &c. They have also been called ' endoplasmatic ' and ' ectoplasmatic ' by Braun and Nodake (1924) and Hofmeier (1927).

In the same paper Weil and Felix (1917) described three types of colonies developing on agar planted from old broth cultures of X strains : (1) colonies small at first, iridescent and later becoming larger and slimy, but with no progressive filmy outgrowth (' O ' colonies) ; (2) colonies with typical filmy growth (' H ' colonies) ; (3) intermediate drop-like colonies which showed the beginnings of filmy outgrowths from their margins.

Immune sera of rabbits prepared from ' H ' and ' O ' X-cultures showed that there were two types of agglutinin developed. The O agglutinin agglutinated the O emulsion in small grains, and the H emulsion in fine flakes, and had no action on the saprophytic strains, while the H agglutinin agglutinated the H emulsion and the saprophytic strains in coarse flakes. The O immune serum contained only the O agglutinins.

The H immune serum contained the O and H agglutinins, the latter by the quicker formation of coarse flakes obscuring the presence of the slower working O agglutinin.

Absorption experiments showed that the O forms possessed one and the H forms two receptors, thus : (1) O immune serum saturated with O or H emulsions was completely deprived of its agglutinins ; (2) H serum saturated with H agglutinated neither H, O, nor the saprophytic strains, the latter possessing receptors identical with the H receptors ; (3) H serum saturated with O strains had still action on H and the saprophytic strains ; (4) H serum saturated with one of the saprophytic strains of Group III lost its agglutinins for these strains, but, still possessing O agglutinin, agglutinated H emulsion in fine flakes. Weil and Felix also found that the O agglutinins like those of typhus-fever patients' sera were less resistant to heat than H agglutinins. Later (1918) they showed that the usual *Proteus* strains possessed O and H receptors and that their O receptors were in all cases distinct from those of the X strains.

Felix and Mitzenmacher (1918) confirmed the presence of O and H receptors in *Proteus* strains, employing and modifying Sach's (1918) method of destroying the H antigen by heating to 80° C. and also Braun's (1918) method of obtaining the O forms by cultivation on carbolic agar.

A Soluble Specific Substance obtained from HX19.

Przesmycki (1926) extracted from cultures of H X19 but not from O X19 polysaccharides which in a dilution of 1 in 10,000 to 1 in 20,000 yielded precipitates with specific anti-H sera.

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Bacillus salmonicida.

B. salmonicida Emmerich & Weibel, 1894, Syn. *B. truttæ* Marsh, 1902.

This is a well-defined bacterium somewhat resembling the large group of proteus-like organisms found in river waters. It produces a distinct form of disease, the 'furunculosis of the salmonidæ'. This manifests itself by necrotic softened areas in the muscles and subcutaneous tissues accompanied by an intense septicæmia, and is often the cause of a high mortality among trout and salmon in Europe (Emmerich and Weibel, 1894). Since 1911 it has been observed in England (Masterman, 1912 and Arkwright, 1912), and in 1926 was recorded in Scotland (Williamson, 1928).

Characters. It is a small, non-motile, Gram-negative, aerobic, non-sporing bacterium usually $7 \cdot 0 \times 1 \cdot 0$ or $1 \cdot 5\mu$ in size, but with some longer forms in old cultures. It grows best at 10 to 20° C., and not at all at 37° C. Growth on agar is in small slightly opaque colonies, which are more luxuriant if serum or blood (mammalian) are added to the medium. Gelatin and inspissated serum are liquefied. Glucose and mannitol peptone water are acidified with gas production. Cane-sugar, lactose and dulcitol are not affected. Milk is turned acid and digested. On agar and gelatin and in broth a very characteristic dark brown coffee-coloured pigment is slowly produced which diffuses into the medium. Sea-water, pure or diluted 1 in 2, is fatal to the bacterium in less than 24 hours.

The bacteria are found in pure culture in the lesions, blood and internal organs.

Members of the salmonidæ and some other fresh-water fish, e.g., gold-fish gobies, can be experimentally infected by inoculation or by adding culture to the water. These fish sometimes become infected but remain in apparent health and act as carriers for two months or longer (Horne, 1928).

Clayton (1927) infected by inoculation plaice, blennies and a codling, and found that the first might retain infection in salt water for 18 days. It seems probable that the reservoirs of endemic disease between the epidemic seasons, which are mainly in the summer, are to be looked for in infected fresh-water fish acting as carriers in rivers, and the disease may be imported with apparently healthy fish from hatcheries. It is not unlikely that infected salmon remain carriers through the winter and spread the disease by ascending fresh rivers the following year.

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B. salmonis pestis.

B. salmonis pestis has been described as the initial cause of the well-known salmon disease associated with Saprolegnia by J. Hume Patterson (1903). It is an aerobic, Gram-negative, motile, non-sporing short bacterium. It grows scantily on potato and liquefies gelatin. Milk is turned acid and digested. Inspissated serum is not digested.

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The Fluorescens-Pyocyaneus Group.

THE GROUP AS A WHOLE.

Chief Characters.

The *fluorescens-pyocyaneous* group includes those species which occur as rods, in size usually 0·5 by 2·0μ; which are usually actively motile, with one or more polar, rarely peritrichous flagella, and are Gram-negative, non-sporing and non-capsulated; which liquefy gelatin, digest milk and coagulated albumin as a rule, grow on agar as a spreading layer, cause turbidity and the formation of a pellicle in broth, form little or no indole, and in suitable media create a yellowish-green fluorescent pigment which is accompanied in many strains by a blue pigment, pyocyanin.

The Relationship of the Group and of its Members.

In causing liquefaction of gelatin and digestion of coagulated albumin the group shows close affinity with that of *B. proteus*. Its action on carbohydrates is much feebler and as a rule the flagella are polar and not peritrichous. The chief characteristic of the group is the capacity to produce a water-soluble fluorescent pigment—fluorescin—which diffuses through the culture medium, colouring it green, blue or yellowish green. Typical specimens of *B. pyocyaneus* in addition produce a blue pigment—pyocyanin—which is insoluble in water but soluble in chloroform. As other pigments are produced by certain members and as some strains of *B. pyocyaneus* in culture lose their capacity to form not only pyocyanin but even fluorescin, difficulties arise in deciding whether a bacillus belongs to the group at all, and whether it is to be classified as a degenerate *B. pyocyaneus* or a *B. fluorescens*; especially is this the case when its history is incomplete. In water some of the commonest saprophytes are *B. fluorescens liquefaciens* and *B. fluorescens nonliquefaciens* and these are regarded by some as strains of *B. pyocyaneus* which have lost their powers of forming pyocyanin. At any rate, these organisms are so closely related that it is convenient to consider them as belonging to one group—the *fluorescens-pyocyaneus* group.

Bergey (1926) includes this group in Genus IV, *Pseudomonas* Migula, of which the type species is *B. pyocyaneus*, designated by him *Pseudomonas æruginosa* (Schröter) Migula. Other members of the group which

will be briefly described here are *B. fluorescens liquefaciens*, *B. fluorescens nonliquefaciens* and *B. proteus fluorescens* Jaeger, which under Bergey's nomenclature appear as *Pseudomonas fluorescens* Migula, *P. nonliquefaciens* and *P. jaegeri* Migula respectively.

Criteria of Differentiation.

Cultural tests. As already mentioned, the chief common characteristic is the production of fluorescin, and in regard to this variation occurs in strains of all the members. The optimum temperature for growth for those isolated from disease conditions, and for *B. pyocyaneus* especially, is higher than that for the fluorescent bacteria living a saprophytic life in water.

Fermentations. On carbohydrates little or no action occurs. The chief enzyme is a protease which digests gelatin, fibrin, coagulated egg albumin, &c. This property is fairly constant and persistent, but occasionally is lost in cultivation even in strains of *B. pyocyaneus*. In *B. fluorescens nonliquefaciens* it is absent.

Serological reactions. In *B. pyocyaneus* group there is a very large number of serological types, and no doubt the same applies to the fluorescent members also when agglutinins are used as a basis of classification. Notwithstanding the variety of types the agglutination test shows close relationship of the various members of the group.

Pathogenicity. The great majority of the members are saprophytes and devoid of pathogenicity ; but *B. pyocyaneus* and some others regardless of their origin can cause toxic symptoms when injected into laboratory animals. In disease conditions of man it is most frequently *B. pyocyaneus* that is found, but occasionally one encounters strains that appear to belong to the fluorescent type and a doubt arises as to whether they are degenerate strains of *B. pyocyaneus* that have lost the capacity of producing pyocyanin or whether they are pathogenic fluorescent strains.

B. PYOCYANEUS.

History.

By W. Bulloch.

The existence of greenish-blue discoloration of surgical dressings was known to surgeons for a long time, and there were many theories to account for it. C. Sédillot (1850), the French surgeon, showed that the blue discoloration was transferable and the idea gradually gained ground that it was a vital process. The French chemist Fordos (1860) made a study of the chemistry of the process and was able to isolate a crystalline substance which he named ' pyocyanine '. His results were confirmed by A. Lücke (1862), who not only showed that the condition was infective, but also found in all the cases examined minute rod-shaped organisms or ' vibrios ', which he considered to be probably the source of the pyocyanin. More accurate bacteriological and cultural studies were made by C. Gessard (1882), who obtained growths of an organism since known as *Bacillus*

pyocyaneus. Gessard spent his life in the study of this organism and added a great many new facts about it down to 1925. The early idea that *B. pyocyaneus* was a harmless curiosity was dispelled by Ledderhose (1888) and Charrin (1889), who clearly demonstrated that it has pathogenic properties—a fact which has been widely confirmed by all subsequent workers. W. B.

Description and Differentiation.

B. pyocyaneus is an actively motile, proteolytic, non-sporing Gram-negative bacillus, chiefly found in the pus of old sinuses, where, as in culture media, it often produces a blue pigment—pyocyanin—and also a fluorescent pigment—fluorescin.

B. pyocyaneus differs from *B. coli* in having little or no action on carbohydrate and in being proteolytic, in the latter point resembling *B. proteus*. In many characters the bacillus shows a kinship to *B. fæcalis alcaligenes*, but the formation of pyocyanin and fluorescin, and the presence of an active protease render the distinction easy when one is dealing with typical specimens of each species.

Morphology ; Cultural Characters ; Lytic Phenomena ; Vitality.

The usual appearance of *B. pyocyaneus* is that of a thin, very actively motile short rod, 0·5 by 2·0μ, though occasionally strains are found which are not so plump but longer. In media containing antiseptics (Charrin, 1889) or urea (Wilson, 1906), long threads with fusiform swellings along their course develop. The motility is due to a single polar flagellum in the majority of strains. Burckhardt (1917) believed that in this there was a means of differentiating *B. pyocyaneus* from *B. fluorescens liquefaciens* and *B. fluorescens nonliquefaciens*, which possess 2 to 5, and 6 to 12 polar flagella. Aoki (1926) amongst his 22 types of *B. pyocyaneus* found 5 which possessed more than 1 flagellum, the numbers being 2, 3, 4, and even on rare occasions 5. Bergey (1926) states that it has 1 to 3 polar flagella. After prolonged cultivation motility may be absent, but can be restored by animal passage.

The bacillus stains readily with the ordinary aniline dyes, and is Gram-negative. Spores have never been found. Capsules are as a rule absent, but Sonnenschein (1927) has seen a normal strain of *B. pyocyaneus* develop into a capsulated form—*B. pyocyaneum mucosum*—in a gall-bladder into which a bacteriophage for *B. typhosus* had been instilled.

Cultivation.

Temperature. The optimum temperature for growth is 37° C., but for pigment production a somewhat lower temperature is favourable.

Reaction. A hydrogen ion concentration of pH 6·6 to 7·0 is most suitable, though growth can occur between the limits of pH 5 to 8.

Oxygen requirement. Growth can occur in the absence of oxygen but is better in its presence. Although the leucobase of pyocyanin can be formed under anaerobic conditions, oxygen is required for the development of the green colour.

Culture media. *B. pyocyaneus* is one of the easiest micro-organisms to grow, no special medium being necessary. On this account it has lent itself to studies designed to elucidate the metabolism of bacteria. For example, Aubel (1921) cultivated it in a solution containing asparagin, magnesium sulphite and acid phosphate of soda, the asparagin being its only source of nitrogen and carbon. It can utilize formates and carbonates as sources of carbon (Braun and Cahn-Bronner, 1921), and in the presence of glucose can obtain its nitrogen from simple nitrates.

In *bouillon* and *peptone water*, uniform turbidity occurs with usually the formation of a thick pellicle which is easily broken up and sinks to the bottom of the tube. The green pigment makes its appearance at the surface and gradually diffuses through the whole medium.

In *gelatin* the surface colonies are large and flat and soon the bacterial mass in the centre is surrounded by a zone of liquid gelatin. The deep colonies are small, and liquefaction occurs only when they reach the surface. In stab culture, growth is best marked at the top, where, as usual, liquefaction and pigmentation first appear.

On *agar* slants the growth is abundant and spreading, at first greyish in colour but becoming yellowish-green to blue after about 18 hours.

Variation and bacteriophage action.

In his work on a transmissible lysis of *B. pyocyaneus*, Hadley (1924) describes the colonies of the ' R ' strain, which is resistant to the action of the lytic element, as round, of limited size, somewhat massive, highly viscous, homogeneous in texture, and forming only fluorescin and no pyocyanin, whilst his ' L' strain formed leaf-like colonies with fimbriate margins, produced much pyocyanin as well as fluorescin, and showed one or more roughly circular corroded or pocketed areas measuring 1 to 5 mm. in diameter, from which the bacteria had largely disappeared, leaving the surface bronze coloured or iridescent. Baerthlein (1918) had already described two types of surface colonies of *B. pyocaneus* on agar : (*a*) a rough, dull, dry, crumbly colony consisting of short, plump bacilli ; and (*b*) a moist, shining, more transparent colony, consisting of slender bacilli.

The action of a lytic agent can profoundly alter the physiological activity of *B. pyocyaneus*. It was Čančik (1923) who was the first to consider the eroded areas where there was a deposit of crystals of pyocyanin as due to the action of a bacteriophage. Whilst Hadley's work was in progress, papers by Blanc (1923), Quiroga (1923), Hauduroy and Peyre (1923) appeared which pointed to similar conclusions. For instance, Blanc found that under the action of lytic filtrates a *B. pyocyaneus* culture was modified so as to yield a growth in which there was suppression not only of pyocyanin but also of fluorescin production, and at the same time there was loss of power to liquefy gelatin. It must, however, be remarked that D'Herelle (1926). Lisch (1924) and Asheshova (1926) do not consider the development of eroded iridescent spots in cultures of *B. pyocyaneus* as being due to a bacteriophage. D'Herelle regards the phenomenon as a

disease of the bacteria resulting in a ' bacterioclysis ', a fragmentation rather than in a dissolution of the bacteria. Hadley (1927) in a paper on microbic dissociation replies to D'Herelle's criticism. Asheshova (1926) has isolated from sewage-polluted water a lytic agent for *B. pyocyaneus* and this he finds has all the characters of a true bacteriophage.

During cultivation, variation occurs without obvious cause. Of 50 strains studied by Aoki (1926) 3 produced fluorescin but no pyocyanin, and 8 produced neither pigment, and as regards liquefaction of gelatin 19 were strong, 18 were weak and 13 were negative in their action.

Smell.

Many strains emit a peculiar odour which has been described at times as aromatic, ammoniacal, or that of linden blossom.

Vitality.

Action of dyes. *B. pyocyaneus* is very resistant to crystal violet, malachite green and brilliant green, and is frequently encountered on media containing these dyes, when efforts are being made to isolate *B. typhosus* from stools. It bleaches many colours, particularly litmus. It develops on MacConkey's bile-salt lactose neutral red agar and is more resistant to the action of bile salts than most strains of *B. fluorescens liquefaciens*. No special medium is required for its isolation, but the use of brilliant green media or the Drigalski medium is helpful.

Viability of cultures. *B. pyocyaneus* is very tenacious of life ; old dried-up agar cultures are often found to contain living germs. Shattock and Dudgeon (1912) found that *B. pyocyaneus* dried on glass slips in air never survived more than nine days, but that in a very complete vacuum vitality persisted for many months.

Autolysis. The bacterial deposit in bouillon cultures in some strains is dissolved with the production of ammonia through the action of a specific ferment. Heating of the cultures to 60° C. destroys the enzyme and prevents autolysis.

Biochemical Reactions.

Action on proteins. *B. pyocyaneus* possesses a proteolytic ferment which at pH 7·2 is very active in the liquefaction of gelatin, the digestion of casein and fibrin, blood-serum and egg albumin. This ferment—a protease —resembles trypsin in acting in an alkaline medium. As regards the effect of heat on it, reports differ, but it would seem to be injured by exposure to 65 to 80° C. for 30 minutes, but even after exposure to 100° C. some action is still manifest. Probably a number of ferments are present in broth cultures and their thermolability varies.

For some years a preparation of the proteolytic ferment named *pyocyanase* by Emmerich and Löw (1899) attracted attention as being of service in the treatment of various infective processes. Pyocyanase was prepared by killing the bacilli in a broth culture of *B. pyocyaneus* by chloroform, filtering to remove the bacteria and then concentrating the filtrate by evaporation *in vacuo*. This product not only digested protein

substances but also the bodies of various bacteria, e.g. anthrax, staphylococci, streptococci, &c. Heating to 100° C. did not destroy its activity. An explanation of this was afforded by Raubitschek and Russ (1909) who showed that heat-resistant lipoidal bodies with a bacteriolytic action could be extracted from pyocyanase. The introduction of pyocyanase into therapeutics was consequent on the observation of Bouchard (1889) that the injection of *B. pyocyaneus* into a laboratory animal which had recently been inoculated with *B. anthracis* frequently arrested the infection with the latter organism. Similar experimental results were obtained by several observers and a number of human diseases were treated by vaccines of *B. pyocyaneus*. Notwithstanding many reports of the beneficial action of pyocyanase it would now seem to be seldom employed. For the solution and removal of sloughs from old ulcers and sinuses it was said to be effective and to have the advantage over various chemical agencies in that it was harmless to granulation tissue. It has also found a use in the treatment of diphtheria with the object of destroying the bacilli and the membrane.

Indole, if formed at all, is produced in only small amounts in culture, but recently Sherwood, Johnson and Radotincky (1926) state that all of the 22 strains examined by them produced indole.

Action on carbohydrates. No observer has reported the formation of gas from any carbohydrate, and the majority have found that no acid is ever produced. Trommsdorff (1911) has seen some strains producing acid from glucose. No doubt the fermentation of a sugar may be masked by the active proteolysis and alkali formation which accompany the growth of *B. pyocyaneus*. Strains examined by the writer have produced acid from glucose. Exact biochemical determinations would probably show some consumption of sugars. Glycerin is very active in favouring pyocyanin formation and one would expect it to be utilized in the metabolism of the bacillus.

Action on milk. Most frequently without previous clotting the milk is peptonized and coloured green. Aoki (1926) in his study of 50 strains found that 30 peptonized the milk without clotting, 13 neither peptonized nor clotted, and a few peptonized and also clotted. The same observer found that litmus milk was slightly reddened at the end of 20 hours, after which it became strongly alkaline with a blue pellicle.

Hæmolysin. Bulloch and Hunter (1900) were the first to observe that filtrates from old broth cultures of *B. pyocyaneus* caused hæmolysis of the red cells of man and of various species of animals. They considered that the filtrates after heating for about 15 minutes at 100° C. became inactive. Others confirmed the observation as to the hæmolysis, but found that heating to 100° C. did not prevent its occurrence ; the latter observation and the fact that no antihæmolysin could be prepared suggested to some that the hæmolysis was not due to hæmolysin, but to the alkali produced in the medium. The work of Landsteiner and Raubitschek (1908) and of Fukuhara (1909) has thrown considerable light on the problem. We now

know that there are two classes of hæmolysin : (1) a genuine hæmotoxin which is thermolabile and for which an antihæmolysin can be prepared ; (2) a thermostable hæmolysin which is soluble in alcohol and ether and is of a lipoid nature and which does not lead to the development of anti-hæmolysin when injected into animals. Moreover, it does not cause hæmolysis *in vivo*, a result to be expected from the fact that its action is arrested by the presence of normal serum.

Pigment Formation.

Pyocyanin.

The power of producing pyocyanin is the one characteristic which, in the present state of our knowledge, distinguishes *B. pyocyaneus* from the fluorescent bacteria which are widely distributed in nature—*B. fluorescens liquefaciens* and *nonliquefaciens* (*B. putidum*). All these bacteria under normal conditions produce a pigment fluorescent and yellowish-green in colour which is soluble in water but insoluble in chloroform, and which on treatment with acids yields a colourless compound to which the colour can be restored by the addition of alkalis. As a standard of differentiation the production of pyocyanin by a bacillus is not altogether satisfactory, as it depends on a great many factors, and under certain conditions, as we have already seen, genuine strains of *B. pyocyaneus* may not only lose their capacity for producing pyocyanin but even for producing fluorescin, whilst retaining their other cultural characters, their characteristic odour and their specific protease. Pyocyanin is non-fluorescent, is bright blue in colour, and crystallizes as fine needles containing ten molecules of water of crystallization. It is insoluble in water but soluble in chloroform, and this reagent is usually employed to extract it from cultures. On the addition of acid it yields a bright red pigment—acid pyocyanin—which is insoluble in chloroform and which can be reconverted into pyocyanin by the addition of alkalis. The chemical composition of pyocyanin has been investigated by numerous workers and different formulæ have been given. The more recent work of Wrede and Strack (1924) and of MacCombie and Scarborough (1923) assign to pyocyanin compositions of $C_{26}H_{24}N_4O_2$ and $C_{26}H_{28}N_4O_3$ respectively.

The development of pyocyanin is influenced by many factors :

1. *Temperature.* As in the case of many other pigment-forming bacteria *B. pyocyaneus* forms pyocyanin in greater amount and earlier at room temperature than at 37° C.

2. *Reaction.* The optimum pH is said to be from 7·4 to 7·8.

3. *Composition of medium.* This has been extensively studied by Gessard (1920). He has shown that meat broth consisting of 1 part of fresh meat and 2 parts of water frequently allows of the development of fluorescin only, in strains which in peptone water or glycerin agar readily form pyocyanin also.

4. *Symbiosis.* Cultivation of *B. pyocyaneus* with *Oidium lactis, M. tetragenus*, streptococci, &c., leads to loss of pigment production.

5. *Bacteriophage action.* As already mentioned, contact with a bacteriophage may result in the production of resistant strains of *B. pyocyaneus* which cease to produce pyocyanin.

6. *Action of carbohydrates.* It has been shown by Emrys-Roberts (1915) that strains which have lost their capacity to produce pyocyanin can have this restored by cultivation in glucose media.

7. *Aerobiosis.* No pigment is produced in the absence of oxygen. In old cultures of *B. pyocyaneus* a red-brown pigmentation is often found and has been attributed by Gessard to the production of a special pigment, whilst others regard it as a derivative of pyocyanin through the action of an enzyme. Gessard (1919 and 1925) has also described: (*a*) *variété érythrogène* strains which produce a brilliant red pigment in such quantity as to mask the traces of pyocyanin in the culture; (*b*) strains producing a black pigment, melanin.

Pyorubrin.

A bright red pigment, said to be characteristic of *B. pyocyaneus*, has been named pyorubrin by Meader, Robinson and Leonard (1925), and has been studied by them. It is soluble in water, insoluble in chloroform, non-fluorescent, and remains unaffected in colour when treated with acids or alkalis. The same investigators show that practically all fluorescent bacteria which are culturally identical with *B. pyocyaneus* will produce both pyocyanin and pyorubrin on appropriate media. Of 10 stock strains of *B. fluorescens liquefaciens* and freshly isolated strains of fluorescent bacteria, 6 produced both pyocyanin and pyorubrin, while of the remaining 4, 3 were old stock laboratory cultures which had almost completely lost their chromogenic functions. The chief purpose of their paper was to demonstrate that this red pigment pyorubrin is characteristic of *B. pyocyaneus* and entirely separate and distinct from the equally characteristic pyocyanin and the non-specific fluorescent pigment.

Toxin Formation.

Charrin (1889) and Wassermann (1896) demonstrated the presence of a toxin in broth cultures and found this still active after passage through a Chamberland filter. Charrin found guinea-pigs, rabbits and pigeons susceptible to its action. Wassermann found guinea-pigs most susceptible, and that an old broth culture in which the bacteria had been killed by toluol in intraperitoneal doses of 0·2 to 0·5 c.cm. caused their death in 6 to 12 hours with symptoms of collapse, dyspnœa and depression of bodily temperature.

Most observers believe that both endo- and exotoxins are present in cultures.

Serological Reactions.

Antitoxin. Wassermann (1896) by injection of goats with filtrates prepared an antitoxic serum of which 0·3 to 0·5 c.cm. neutralized 10 lethal doses for guinea-pigs.

Antienzyme. Several investigators have prepared an antienzyme for the protease. More recently, Launoy (1920) has confirmed these earlier results and has concluded that the injection into an animal of a filtrate of a proteolytic organism results in the production of antibodies against the proteolytic ferment and that these antibodies are specific for the particular bacterial species against which the animal was immunized. Dukes (1922), however, found that anti-ferments are not produced against the proteolytic enzymes of *B. pyocyaneus*, and that the inhibition found in immune serum by some methods of testing appeared to be due to the action of a precipitin.

Agglutinins. The agglutinins in the sera of rabbits immunized against strains of *B. pyocyaneus* show great diversity. The most complete investigation of the classification of *B. pyocyaneus* on serological grounds has been made by Aoki (1926), who made a careful study of 50 strains the cultural characters of which were at the same time investigated. He prepared 37 immune sera and found that the 50 strains could be placed in 22 types, designated by the letters of the alphabet—a, b, c, &c. Many of the types contained only a single strain, but among the larger were a, b and c, which contained 7, 9 and 10 respectively. The divisions based on agglutination, often, but by no means invariably, corresponded with those based on cultural characters. Strains were included in the study which on cultural grounds could not be distinguished from *B. fluorescens liquefaciens* and *B. putidum.*

Serological Relationship of B. pyocyaneus *to fluorescent bacteria.* Trommsdorff (1916) has shown that the fluorescent bacteria are heterogeneous in their response to agglutinins. Pribram and Pulay (1915) found some cross agglutination between members of the *B. pyocyaneus*, *B. fluorescens*, *B. proteus* and *Vibrio* groups. A further possible indication of the relationship of *B. pyocyaneous* to *B. proteus* is the fact that the serum of typhus-fever cases often agglutinates both certain strains (' Z ' strains) of *B. pyocyaneus* (Neukirsch and Kreuscher, 1910; Wilson, 1922) and X19 strains of *B. proteus*, and according to Friedberger, Zorn and Meissner (1922) the receptors of ' Z ' are found in the *Proteus* strains and the receptors of the X19 in the ' Z ' strains.

Agglutinins in human infections. The titre of normal blood for *B. pyocyaneus* seldom exceeds 1 in 20. In infection with *B. pyocyaneus* agglutination in dilutions from 1 in 40 to 1 in 40,000 is known to occur ; frequently the titre is 1 in 100 to 1 in 500 and more rarely 1 in 1,000.

Pathogenic Action.

In animals.

Gessard, Charrin and others, as soon as pure cultures were obtained, found that laboratory animals—guinea-pigs, rabbits, mice and pigeons—could be infected. In subcutaneous inoculation there follows œdema, necrosis of the skin and wasting, often terminating fatally. Wassermann found that 1/10 *öse* of the growth from a virulent culture on agar on

intraperitoneal injection caused the death of a guinea-pig inside 24 hours. On post-mortem examination, peritonitis with a hæmorrhagic exudate was found. Applied to the eye of rabbits, *B. pyocyaneus* causes ulceration of the cornea and iritis.

Occasionally, instances of spontaneous infection of animals are met with, and the micro-organism is believed by some to be the cause of dysentery in calves, and of rhinitis and meningitis in young pigs.

In man.

Not infrequently *B. pyocyaneus* is found in old wounds, in discharging sinuses, and in otitis media. It is more often a secondary invader than a primary excitant of inflammation. Where it is the primary cause of an infective process, the subject is usually young or debilitated (Williams and Cameron, 1896). In an epidemic of umbilical infections of new-born children, Wassermann found the agent of infection to be *B. pyocyaneus*. Lesions of the skin chiefly in the neighbourhood of the umbilicus have been described, and *B. pyocyaneus* has been incriminated. This condition has been named *ecthyma gangrænosum* (Hitschmann and Kreibich, 1897) and is characterized by the presence of bullæ and of hæmorrhagic exudates. When the bullæ burst, ulcers with little tendency to pus formation result. The condition is frequently fatal. As to whether the bacilli enter the skin by local penetration or are carried there as metastases is undecided. The same applies to a necrotic inflammation of the stomach and intestines, which has been attributed to the action of the bacillus.

Generalized infection. In adults a condition resembling typhoid fever, due to infection with *B. pyocyaneus*, has been described by numerous workers. A characteristic feature of a generalized *B. pyocyaneus* infection is a tendency to hæmorrhage, e.g. in skin, lungs, bowel and meninges. There is usually fever, dyspnœa, diarrhœa, vomiting, cramp in the muscles, enlargement of spleen and often of the liver. Many cases end fatally. At post-mortem examination, swelling of Peyer's patches, necrosis of mucous membrane, and ulcers resembling those produced by *B. tuberculosis* are frequently found. Enlargement of spleen and hæmorrhages into subcutaneous and submucous tissues are frequent.

Dysentery. The causal relationship of *B. pyocyaneus* to enteritis and dysentery is reviewed by Justi (1915), who concludes that *B. pyocyaneus* in the intestine is usually harmless, but Calmette (1892) and Lartigau (1898) record cases of dysentery where it was the infective agent.

Infantile diarrhœa. In some epidemics of this condition *B. pyocyaneus* has been found in the stools, but it probably is not the primary cause, since in typhoid fever it is not unusual to find *B. pyocyaneus* accompanying *B. typhosus*.

Meningitis. In a generalized infection *B. pyocyaneus* may attack the meninges and cases are on record where it has caused the disease by its presence in cocain solution introduced for anæsthetic purposes.

Ulceration of the cornea (Garretson and Cosgrove, 1927) and *diminished motility of stomach and intestine* (Ashby, Eldridge and Freeman, 1927) have been attributed to the action of *B. pyocyaneus*.

Cystitis. Aoki and others refer to the source of certain of their strains as being derived from the urine. Klieneberger (1907) found *B. aerogenes* and *B. pyocyaneus* associated in a case of cystitis. Recently the writer has met with three cases of cystitis where *B. pyocyaneus* was the only infecting agent present. Two of the patients were pregnant and the condition cleared up under medical treatment.

Habitation and Viability of the Bacillus.

As *B. pyocyaneus* is found occasionally in the healthy intestine of man and other animals and is frequently found where there is diarrhœa or enteritis, its presence in contaminated water, soil and sewage can be accounted for. Possibly it can adapt itself to a purely saprophytic mode of existence. It is one of the most resistant of non-sporing organisms against the bactericidal action of light, heat and desiccation. Burge and Neill (1915) found that fluorescent bacteria are better able to resist ultra-violet light than those which do not produce this pigment. In the human body it is frequently found on the skin in the region of the perineum.

Natural Resistance of Man and Animals.

As a rule, *B. pyocyaneus* is not capable by itself of infecting the body. While the skin and mucous membranes of the body are intact it is a mere saprophyte on their surfaces. When lesions of these surfaces occur as a result of traumatism or infection with other bacteria, *B. pyocyaneus* can multiply in the serous exudates and no doubt aggravates the condition. When *B. pyocyaneus* is the primary infecting agent it is the rule to find that the hosts are very young or are in a debilitated condition, but exceptions occur and possibly strains of greater virulence arise and are gifted with greater invasive power. Normal serum is antagonistic to the hæmolysin and possibly to the other toxins of the bacillus.

Practical Diagnosis.

The formation of pigmented colonies renders easy the detection of the presence of *B. pyocyaneus* in pus, urine and other fluids and exudates. To isolate it from fæces it is an advantage to employ the media that are of service in connection with the isolation of *B. typhosus*.

Immunization.

Injection of dead cultures and filtrates of *B. pyocyaneus* were at one time employed on account of their antagonistic effect on the development of other bacteria, e.g. *B. anthracis*. A few cases are on record of the treatment of chronic sinuses containing *B. pyocyaneus* with vaccines.

Green Fluorescent Bacteria from Water.

Very closely related to *B. pyocyaneus* are the fluorescent bacteria which are among the commonest of water micro-organisms, and which

as regards their action on gelatin, are commonly known as *B. fluorescens liquefaciens* and *B. fluorescens nonliquefaciens*. The latter is also designated *B. fluorescens putidus* or *Bacterium fluorescens putidum*. In a study of 100 fluorescent strains isolated from water, Tanner (1918) found that all were Gram-negative and motile, possessing usually a tuft of flagella, though occasionally a strain with one flagellum is encountered. None of them formed gas in any carbohydrate medium and all produced acid in glucose, but no fermentation of saccharose and lactose occurred. None of them formed indole. About one half of them liquefied gelatin, peptonized casein, reduced nitrates to nitrite and ammonia, and formed hydrogen sulphide. An absence of diastatic action upon potato starch was noted. All of them grew well at 20° C. and at 37° C., and all produced a green diffusible pigment.

Bergey (1926) distinguishes 20 species of the *Pseudomonas*, his classification being largely based on their actions on gelatin, position of flagella, coagulation of milk, formation of indole and reduction of nitrates. According to him *B. pyocyaneus* liquefies gelatin, coagulates and peptonizes milk, reduces nitrates and forms indole, whilst *B. fluorescens liquefaciens* although liquefying gelatin and reducing nitrates does not form indole nor coagulate milk. *B. fluorescens nonliquefaciens* has peritrichous flagella, has no action on gelatin and milk, does not reduce nitrates and does not form indole. From our study of *B. pyocyaneus* it is obvious that apart from the formation of pycocyanin there is no cultural difference between it and *B. fluorescens liquefaciens*.

We have already discussed the relationship of the various members based on agglutination.

Bergey describes as a separate species *Pseudomonas jaegeri* Migula, which had been isolated from Weil's disease and from water by Jaeger (1892), who regarded it as a member of the *Proteus* group capable of producing fluorescin, and who gave it the name *B. proteus fluorescens*. It is now known not to be the primary infective agent in Weil's disease, with which it may be associated in much the same way as the ' X ' strains of *B. proteus* are associated with typhus fever.

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CHAPTER V. THE CHOLERA VIBRIO AND RELATED ORGANISMS.

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History of Asiatic Cholera.

By W. Bulloch.

Since Asiatic cholera began to appear in the beginning of the nineteenth century, a vast amount has been written on every aspect of the disease. The historical and epidemiological sides of the question have been treated with great thoroughness by Hirsch (1881) and by Sticker (1912). The ætiology remained, however, for long a mystery. It was during the fifth pandemic of the disease that a German Commission, consisting of R. Koch, Gaffky and Fischer, was sent to Egypt to study the disease. They began their investigations in Alexandria in September, 1883, and by the time they had examined 10 cholera autopsies, they had come to the conclusion that the blood and tissues were sterile. In the intestinal contents, and particularly in the intestinal wall, and lying for the most part superficially they found, however, what was regarded as a special bacillus. On account of the cessation of the epidemic in Egypt, Koch and his co-workers went on to India, and were installed in the Medical College Hospital in Calcutta in December, 1883. Here it was that the main facts of the ætiology of cholera were discovered by Koch, after a thorough study of 42 living cases of cholera and 28 autopsies. Altogether he examined close on 100 cases of the disease in Egypt and India. On closer inspection, the micro-organism found first in cholera in Egypt and then in India proved to be definitely curved like a comma (' Eine Komma ähnlich '), and this became almost universally known as the ' comma bacillus ' and later as the ' Vibrio choleræ '. The early accounts of Koch's work were published in a series of seven reports in the *Deutsche medicinische Wochenschrift*. (1883, 1884), and much fuller information was given by Koch at two Conferences called at Berlin to discuss the cholera question. The first of these conferences was held in July, 1884, and the second in May, 1885. In the first conference will be found the fullest account of Koch's work on the comma bacillus. He gave an accurate description of its appearance, its motility and its distribution in the body. He found that it was essentially aerobic, and grew at between 16 and 40° C. He could find no evidence of spores. He described in great detail the cultural

characters of the bacillus on gelatin plates, potato and other media. He also noted the fact that it may dissolve blood corpuscles. At the second conference he gave a detailed account of his attempts to produce cholera experimentally in guinea-pigs. From the constant presence in cases of cholera, both during life and after death, from the special characters of the organism itself, and from its pathogenic effects in animals he concluded that the comma bacillus is the cause of Asiatic cholera. The announcement of his discovery was regarded with world-wide interest, and his statements were soon confirmed by Babés (1884), by Watson Cheyne (1885) in Paris, by Nicati and Rietsch (1885) and by van Ermengem (1885), who studied the disease in Marseilles.

On the other hand, Koch's conclusions were adversely criticized by Timothy Lewis (1884) and especially by E. Klein (1885), who headed a commission from England to study the disease in India. Roy, Brown and Sherrington (1887), working in Madrid (1885), were also unable to accept Koch's views on the ætiology of cholera. Koch also had a strong opponent in Emmerich (1884, 1885), of Munich, who investigated the disease in Naples. Vibrios like Koch's comma bacillus were also found in non-choleraic material. Two of these vibrios, namely those of Finkler and Prior (1884) and of Deneke (1885) figure largely in the early cholera literature. By degrees, however, Koch's standpoint gained ground and came to be almost universally accepted, for wherever cases of cholera were examined by proper bacteriological technique, the comma bacillus was found, and the true comma bacillus was not found in material unassociated with cholera. The discoveries of the existence of cholera-like vibrios began, however, to loom largely in the literature as the result of observations of Gamaléia (1888), of D. D. Cunningham (1891), Pasquale (1891), Ivanoff (1893), Sanarelli (1893), Dunbar (1895, 1896) and others, and a great amount of work was done in attempts to differentiate the ' cholera-like ' from the true cholera bacterium. New tests were devised. Among these the ' cholera red ' reaction of Bujwid (1888) and Dunham (1887) held sway for a short time and was explained by Salkowski (1887).

During subsequent years the cholera literature grew apace, and it received great accessions following the huge epidemic of cholera which occurred in Hamburg in August-September, 1892, when 18,000 cases and 8,200 deaths occurred in this city in a few weeks. In other European countries there was also a wide dissemination of cholera. The early diagnosis of the disease was improved by the application of the enrichment method of culture by Dunham (1887) and applied particularly by Koch (1893), who laid down very exact rules for the bacteriological diagnosis of cholera according to the knowledge of the time. The differential bacteriological diagnosis made rapid strides through the application of immunological reactions, and here must be mentioned especially the researches of R. Pfeiffer beginning in 1894, and continued for several years. Pfeiffer's work has proved of paramount importance. Diagnosis by agglutination methods was applied in cholera by Achard and Bensaude

(1896). In the domain of prophylaxis by inoculation of cholera cultures Ferran (1885) has claimed priority, even if it is now admitted that his cultures were impure. The principal exponent of cholera inoculation was W. M. Haffkine, who from 1893 onwards inoculated vast numbers of people in India with cholera vaccine.

The Group of Vibrios and Spirilla—Classification and Nomenclature—General Biological Characters of the Cholera Vibrio—Relationship to Allied Organisms.

By T. J. Mackie.

The Group of Vibrios and Spirilla.

The *Vibrio choleræ* of Koch (*Vibrio comma* Schroeter), the specific organism of Asiatic cholera, may be included in bacteriological classification among the *Spirillaceæ* (Migula, 1894) recognized as a family of the *Eubacteria* or *Eubacteriales* (classification of the Society of American Bacteriologists ; see Winslow *et al.*, 1917). The family name *Spirillaceæ* was apparently used first by Migula, and was adopted by Fischer (1897) and by later systematists (see Buchanan, 1925). Migula recognized as constituent genera, *Spirillum*, *Spirosoma*, *Microspira* and *Spirochæta*. Fischer described the genus *Vibrio* as referable to the *Spirillaceæ*. In the classification of the Society of American Bacteriologists, two genera are included : *Vibrio* and *Spirillum*, differentially characterized as follows : *Vibrio*—short, bent, rigid, rod-shaped organisms ; occurring singly or united into spirals ; motile by means of a single terminal flagellum (or rarely two or three flagella) ; gelatin-liquefiers and active ammonifiers (in many species) ; aerobic and facultatively anaerobic ; not producing endospores ; usually Gram-negative ; mostly water saprophytes but a few species parasitic ; type species *Vibrio choleræ* Koch (Winslow *et al.*, 1917) ; *Spirillum*—rigid, rod-shaped organisms of various thicknesses, length and pitch of spiral, forming either long screws or portions of a turn ; motile by means of multiple flagella, at one or both poles ; stated in some species to produce endospores ; habitat : water or putrid infusions.

Certain non-motile, non-flagellate, comma-shaped organisms have been described in the literature ; to these Migula originally applied the name *Spirosoma*. The question arises, therefore, whether such organisms should be included in the genus *Vibrio*. The vast majority of Vibrio types which have been described are flagellate and motile, but loss of flagella and motility is an undoubted variation which certain vibrios, e.g., *V. choleræ*, may undergo (vide p. 348). It would seem justifiable, therefore, to extend the possible generic characters so as to include non-motile forms. It is obvious that a sharp line of distinction can hardly be drawn between vibrios and spirilla. A further difficulty arises in classification : organisms which would generally be designated as *Bacillus fæcalis alkaligenes* are found to possess terminal flagella and show a tendency to vibrionic form. In fact,

B. fæcalis alkaligenes has been classified by some writers as a vibrio (vide p. 434). It is apparent also that straight, rod-shaped forms possessing a single terminal flagellum and designated '*Pseudomonas*' show some affinities with the vibrios.

The vibrios and spirilla have long been recognized in the simplest form of bacterial classification as a morphological group differentiated from others of the lower bacteria (or *Eubacteria*) and characterized as short, curved, cylindrical organisms (*Vibrios*) or as elongated, non-flexuous cells showing a spiral structure (*Spirilla*). As a generic name, the term *Spirillum* was first used by Ehrenberg (1830) to include all the various spiral bacteria. Cohn (1872) also employed this designation. Flügge (1886) applied the name *Spirillum* in a broad sense, and the organisms now classified as *Spirochætes* were included under this head. Other systematists (see Buchanan, 1925) also placed among the spirilla both rigid forms and those flexuous curved or spiral filamentous organisms which would now be entirely separated from the *Spirilla* and classified as *Spirochætes*. Thus, in recent systems of classification the *Vibrios* and *Spirilla* have been differentiated from the *Spirochætes*, one of the essential biological differences being the structural rigidity of the former and the flexuous character of the latter.

The generic designation *Vibrio* was first proposed by Müller in 1773, and in the earlier bacteriological literature the name was used in a somewhat varied application to different biological types. In 1913, Vuillemin suggested that the short, bent, rod-shaped forms should be classified as *Microspira*, and the name was adopted by many writers. Schroeter, in 1886, had proposed this generic designation with Koch's vibrio as the type species. Other names that have been applied to the cholera vibrio in the past are "Komma Bacillus" (Koch), *Microspira comma* (Schroeter), *Spirillum choleræ asiaticæ* (Flügge) (see Bergey, 1926). The designation now accepted by the American systematists is *Vibrio comma* (Schroeter).

Species referable to the Genus *Vibrio* and their Occurrence.

Many species of the genus *Vibrio* have now been recognized and these will be dealt with in appropriate detail in a later section. Some exhibit a remarkably close biological relationship to the cholera organism; certain of these have been isolated from diarrhœal and even choleraic conditions, and various writers have suggested the existence of an intestinal infection, 'paracholera', bearing the same pathological relationship to true cholera as paratyphoid does to true typhoid fever, the causative organisms differing from the cholera vibrio mainly in serological characters. Such organisms have been classified as '*paracholera vibrios*'. Similar organisms have been isolated from the stools of cholera convalescents and healthy persons in Eastern countries and from water supplies. The whole question of 'paracholera' will be discussed later (vide p. 424). It should be noted, however, that the term '*paracholera*' was first applied by Kraus (1909)

to the hæmolytic vibrios otherwise identical with *V. comma*, e.g. the *El Tor vibrio* (vide p. 364). Various types of vibrios have been isolated from the human subject, existing either as commensals or present in pathological lesions, e.g. *V. milleri* (Miller, 1885) from carious teeth, *V. sputigenus* (Brix, 1894) from the sputum of a case of pneumonia, *V. tonsillaris* (Stephens and Smith, 1896) from the throat in cases of diphtheria, *V. wolfii* (Wolf, 1893) from the cervix uteri in a case of endometritis, *V. surati* (Lamb and Paton, 1913) from the blood of a case of endocarditis, *V. tenuis* (Veillon and Repaci, 1912)—an anaerobic organism—from ulcerating lesions in pulmonary tuberculosis.

An organism presenting many similarities to *V. choleræ* was isolated by Gamaléia in 1888 from an outbreak of enteritis in fowls, and apparently similar types have been found in human fæces and in water (vide p. 431). This organism has been designated *V. metchnikov*. The *V. fœtus* described by Smith and Taylor (1919) as a causative organism of infectious abortion in cattle is a further representative of the genus. Certain species derived from the excreta of swine have been described ; most of these are quite non-pathogenic (see Ford, 1927).

A considerable number of vibrio types have been found in water and there is reason to believe that some representatives of the genus are natural water saprophytes. Bergman (1909) isolated a vibrio (*V. anguillarum*) from an infectious disease of eels and a similar organism from an infectious condition of codlings. He suggested the existence of a group of vibrios pathogenic to fishes. David (1927) has recently described a vibrio (*V. piscium*) in a disease of carp. Vibrios have also been isolated from mussels and oysters (Klein, 1905 ; Remlinger and Nourri, 1908).

Other sources of vibrios described in bacteriological literature are cheese (*V. tyrogenus*, Deneke, 1885), and hay infusion (vide p. 435).

General Biological Characters of *Vibrio Choleræ*.

Before considering the relationships of *V. choleræ* to other organisms, its biological characters may be summarized as follows :

Morphology. *V. choleræ* is exceedingly pleomorphic under certain conditions ; the typical form, however, is a curved, rod-shaped organism 2 by $0 \cdot 4\mu$ in average dimensions ; it occurs singly or in spiral chains ; is actively motile and possesses a single terminal flagellum ; Gram-negative.

Cultural characters. Aerobic and facultatively anaerobic ; optimum growth temperature 37° C. ; grows on ordinary media and flourishes on highly alkaline media. Colonies on nutrient agar—circular, white, moist, translucent, becoming slightly brownish in colour. Gelatin-stab culture shows slow funnel-shaped liquefaction. Growth in alkaline bouillon produces a general turbidity with a fragile wrinkled pellicle. Growth on alkaline potato—white, moist, glistening often becoming pigmented, the colour varying, e.g. greyish-yellow, yellow, yellowish-brown, brownish-red, pink. Solidified blood-serum is slowly liquefied. Milk is not coagulated, but is slowly peptonized.

The typical *V. choleræ* is non-hæmolytic, but certain strains are hæmolytic (e.g. the *El Tor vibrio*).

Indole is formed in peptone water and nitrates are reduced to nitrites ; the addition of sulphuric acid to peptone-water cultures produces a pink coloration (' cholera-red reaction '), i.e., the nitroso-indole reaction.

Glucose and various other carbohydrates are fermented, but without gas production.

V. choleræ : Differentiation from, and Relationship to, other Vibrio Species.

The cholera vibrio represents one of many species referable to the genus, and the general biological differentiation of this organism from other species is of considerable interest and practical importance. Serological reactions have generally been accepted as the final criterion of identity of *V. choleræ*, but as will be shown later, vibrios serologically distinct from, but closely related in other characters to, the classical cholera organism are associated also with choleraic disease—the ' *paracholera vibrios* '. It has been suggested that the cholera vibrio may under certain conditions lose its specific serological characters (vide p. 369).

In morphological characters the group of vibrios are remarkably uniform, closely resembling *V. choleræ* in this respect, and the vast majority of the recorded species are actively motile. Certain species have been described, however, which present marked differences from *V. choleræ*, e.g. ' *V. nasalis* ' of Weibel (vide p. 435) described as a non-motile organism approximating in thickness to the anthrax bacillus. Nobechi (1923) has drawn attention to exceptional strains, apparently true cholera vibrios, which were devoid of flagella and non-motile, and Baerthlein (1912), Olsson (1915) and Balteanu (1926) have described non-motile variants of *V. choleræ*. Emphasis has been laid by some observers (Kolle and Gotschlich, 1903) on the mono-flagellate character of *V. choleræ ;* certain vibrios differ in possessing multiple terminal flagella, e.g., the *Massauah vibrio* (vide p. 431).

Differences in the optimum temperature for growth have been noted according to the natural adaptations of the particular species. The cholera vibrio and other vibrios of intestinal origin grow best at 37° C., but purely saprophytic types and vibrios parasitic to cold-blooded animals have a lower optimum temperature and fail to grow at 37° C. or grow feebly at this temperature.

A few types differ from the majority of species in their anaerobic character (e.g. *V. tenuis*, vide *supra*) and contrast with the aerobic *V. choleræ*. Though the cholera vibrio is a facultative anaerobe, growth under aerobic conditions is invariably more pronounced than in reduced oxygen tensions.

Certain vibrios have been found to be markedly phosphorescent in artificial culture (e.g. *V. phosphorescens*, vide p. 433). *V. choleræ* is quite devoid of this property.

Chalmers and Waterfield (1916), in attempting to elaborate a system of classification of vibrio species, emphasized the formation of pigment in peptone water as a feature of a group which they designated '*V. drennani*', the type organism of this group being the vibrio described by Drennan (1914) which produced a dark-brown pigment even when growing in peptone water. Vibrios characterized by marked pigment formation had been described by Weibel in 1888, viz. '*V. aureus*' and '*V. flavus*' both isolated from canal mud. It is questionable to what extent pigment-production serves to demarcate such a group as contrasted with *V. choleræ*. The cholera vibrio growing in peptone water shows, of course, no obvious pigment formation, but on the other hand, chromogenesis may be well marked in potato cultures, and even on ordinary nutrient agar pigmentation is observable. The property of chromogenesis seems a general one in the genus though more pronounced in certain species according to the medium in which the organism is growing.

A noteworthy character of *V. choleræ* is the power of liquefying gelatin and solidified serum, i.e. its proteolytic action; and in this respect the cholera organism differs from certain recorded species, e.g. *V. tonsillaris* (vide *supra*). This is not, however, a fixed character of the cholera vibrio; thus, after continued cultivation it may be lost. Great stress has been laid by various writers on the type of liquefaction of gelatin in stab cultures, and the rate at which it occurs, e.g. in the differentiation of *V. choleræ* and the *V. proteus* of Finkler and Prior (1884) originally isolated from the fæces of a case of 'cholera nostras'. Thus, the former produces usually a funnel-shaped liquefaction, while the latter develops rapid saccate liquefaction. Such difference may be merely a quantitative one, and the same is true for the rate of liquefaction of coagulated serum. The paracholera vibrios described by Mackie and Storer (1918) while closely resembling *V. choleræ* in biological characters differed in their more rapid liquefaction of gelatin and serum, but at the same time strains differed to some extent from one another in this respect. It seems doubtful whether the type of gelatin liquefaction offers a means of establishing any essential biological differences.

A striking biochemical reaction of *V. choleræ* is the 'cholera-red' reaction (vide p. 362); certain vibrios fail to show this reaction (e.g. *V. proteus*), and can be differentiated from the cholera vibrio by this negative character; on the other hand, the effect is not restricted to *V. choleræ*, and the property is not an entirely stable one.

Considerable attention has been paid to the pronounced hæmolytic property of certain vibrios as contrasted with *V. choleræ* and, as is well known, certain strains which are identifiable serologically with *V. choleræ* may be actively hæmolytic, e.g. the *El Tor vibrio* (Gotschlich, 1905).

V. metchnikovi differs from *V. choleræ* in its high virulence for guinea-pigs and pigeons under experimental conditions. Various types differ from *V. choleræ* in their lack of pathogenicity to laboratory animals

e.g., by intraperitoneal injection of guinea-pigs. *V. choleræ*, however, may lose virulence on cultivation.

While various species can be distinguished from *V. choleræ* by general biological characters there remain types which exhibit no essential difference, and serological tests afford the only means of clear demarcation. This category includes certain of the vibrios reported in paracholera, e.g., *V. gindha* originally described by Pasquale (1891) and reported in a paracholera case in 1916 by Chalmers and Waterfield (vide *supra*), the vibrios described by Mackie and Storer (1918) and by Mackie (1922). In the identification of *V. choleræ* agglutination by a specific antiserum, and other correlated specific serum effects (bacteriolysis, complement fixation), have been generally regarded as indispensable criteria. Various writers have claimed, however, that a typical cholera vibrio may lose its serological character, e.g., agglutinability, and conversely that inagglutinable vibrios may acquire the property of reacting to an agglutinating serum, e.g., by passage through animals (Wankel, 1912, and others). This has been disputed, however, by McLaughlin and Whitmore (1910) and others. It has also been suggested that loss of agglutinability may occur in the body (Flu, 1914). Crendiropoulo (1913) noted that agglutinable vibrios in fæces may be succeeded by inagglutinable vibrios in the gall-bladder. Greig (1916) has described a vibrio differing serologically from *V. choleræ*, which after injection into an experimental animal acquired antigenic properties resembling those of the true cholera vibrio. Such observations raise the question whether *V. choleræ*, or particular strains of the species, may be labile in antigenic properties. Recently Tomb and Maitra (1926) have claimed that cholera vibrios lose their specific serological properties by residence in water, and have suggested that agglutinable vibrios become inagglutinable in the intestine of convalescents. The question of such serological variation of *V. choleræ* remains debatable, and will be discussed fully in a later section.

A further question has arisen : whether *V. choleræ* constitutes a serologically homogeneous species ; this has been maintained by most observers (see Douglas, 1921), but some Japanese writers have claimed the existence of two serological types—the ' Koch type ' corresponding to the classical organism and another designated the ' Takeuchi type ' (Kabeshima, 1918 ; see Takano, Ohtsubo and Inouye, 1926). Nobechi (1923) has stated that among *V. choleræ* strains isolated in Japan in 1921, three immunological types could be recognized, two of which correspond to those identified by Kabeshima, the third being an intermediate variety. Inouye and Tatsuo Kakihara (1925) have also recognized three serological types designated ' *V. choleræ I, II and III* '.

The essential characters by which collectively the *V. choleræ* species may be readily distinguished from other members of the genus are : motility, single terminal flagellum, aerobic growth, absence of phosphorescence, absence of pigment in peptone-water cultures, the cholera-red reaction, funnel-shaped liquefaction of gelatin, pathogenicity to

laboratory animals by intraperitoneal and intravenous injection, low virulence for pigeons by intramuscular injection, and the specific serological reactions with homologous antisera.

Morphology and Staining Reactions of Vibrio choleræ.

By T. J. Mackie.

General Morphology.

Though this organism has so frequently been described as presenting a highly characteristic morphology, its polymorphism under certain conditions is an outstanding biological feature, and has led to some speculation regarding a possible life-cycle. Even under optimum conditions *V. choleræ* tends to show some degree of morphological variation. In recently isolated young cultures (after 18 to 24 hours' incubation at 37° C.) on a carefully standardized nutrient agar (e.g., pH 8·0) forms which may be described as typical usually predominate. In the living unstained condition and also when fixed and stained, these appear as short, definitely curved cylindrical organisms, with rounded or slightly tapering ends, and measuring usually 1·5 to 2μ in length by 0·3 to 0·4μ in breadth. The curved appearance has always been regarded as a characteristic feature and originally led Koch (1883) to describe the organism as a 'comma-bacillus'. In addition to the curvature in one plane the organism is slightly twisted. In young cultures longer and slightly thicker individuals may also be seen, attaining dimensions of 4 by 0·5μ, and a certain degree of variation in size is usually observed. While the typical forms show an unmistakable curvature individual organisms may appear almost straight. In microscopic preparations the degree of apparent curvature depends, of course, on the position of the cell: individuals whose plane of curvature is parallel to that of the microscopic field will appear more bent than others lying in different planes. Thus, in hanging-drop preparations it is noted that an individual which may at first appear almost straight, as it moves and rotates on its long axis exhibits an indubitable curvature. Apart from such variations in apparent curvature, individuals differ in the degree of their actual curvature, and relatively straight organisms may be seen. Different strains also may vary in their morphological characters; in some, almost straight forms with only slight curvature may predominate, contrasting with others in which the organisms are mostly short and markedly bent; some strains may exhibit forms so short and so slightly curved as to appear like cocco-bacilli. It may be noted here that dark-ground illumination is a specially suitable microscopic technique for the study of the morphological appearances of this organism and also of its movements (vide *infra*).

While, generally, young recently isolated cultures exhibit the typical morphology, even young subcultures of strains that have been growing in artificial culture for prolonged periods tend to show atypical forms which lack the characteristic curvature of the recently isolated vibrio. On the other hand, animal passage tends to restore the typical appearance.

In young cultures many vibrios occur singly, but some show a tendency to adhere end-to-end. Thus pairs may be seen attached together with their curves alternating (i.e. the so-called ' S ' forms) or with their curves so arranged that they describe a semicircle. In multiplying, the organism elongates and then divides by transverse fission ; thus pairs of vibrios may remain adherent after division. Undivided, somewhat elongated organisms may also be noted, especially in older cultures, with two or more alternating curves or spiral coils. The spiral structure of these is well seen by dark-ground illumination and particularly while the organism is in motion. In older cultures after three days' growth and particularly in fluid media, chains of vibrios may be observed sometimes of considerable length, their curves alternating regularly in spiral formation. Thus the curvature of the individual short vibrionic forms represents a single section, or coil as it were, of a complete spiral. In older cultures also irregular chains of organisms and elongated filamentous forms may be noted without definite spirality, but with irregular curves. In old cultures, distorted and highly atypical forms become predominant ; these may be interpreted as due to involution or degenerative changes and are discussed later.

V. choleræ is a non-sporing organism.

Motility.

When a young culture in fluid medium (or a fluid suspension of a young culture on solid medium) is examined microscopically in the living unstained condition, the motility of the vibrio is very easily detected and is of an exceedingly active type, the organisms darting about in the microscopic field with great speed. In fact, the movement is well described as ' scintillating '. The rate of movement of *V. choleræ* exceeds that of many other motile bacteria. Koch (1883) in his original observations commented on this feature, and Sanarelli (1919) has stated that the speed of the organism is five times that of *B. typhosus*. Sometimes ' centrifuge ' movement is seen, vibrios rotating rapidly on their short axes. Just as the organism, when cultivated artificially for long periods, shows deviation from its typical morphology (vide *supra*) so also movement is less characteristic and less rapid. Motility is due to a single polar flagellum (as originally described by Koch), which can be demonstrated in specially stained preparations, though, of course, not visible by the ordinary microscopic technique. Various early writers, however, alluded to the occurrence of multiple flagella (see Kolle and Prigge, 1927), but before the introduction of accurate identification of *V. choleræ* by serological methods. It seems unlikely that these polyflagellate organisms were true cholera vibrios. Kolle and Gotschlich (1903) examined 60 strains of serologically true cholera vibrios isolated in Egypt and established the monoflagellate character of the organism, whereas certain non-cholera vibrios isolated from fæces undoubtedly possessed multiple terminal flagella, varying in number from two to six (vide p. 427). The monoflagellate character has been generally confirmed (Greig and others).

The occurrence of two flagella, one at each pole, may be observed very occasionally in single individuals (see Balteanu, 1926). Nobechi (1923) has reported two non-motile strains of *V. choleræ*, otherwise perfectly typical; immobility was regarded by him as due to loss of the flagellum and persisted in culture during a period of 30 months' observation. One of these strains had been recovered from a patient, the other was a 'type culture' from the Koch Institute in Berlin. In this connection it is noteworthy that Baerthlein (1912) and Balteanu (1926) have described a *V. choleræ* variant characterized by its opaque colonies and the non-motility of the units. Balteanu (1926) found that the individuals of the opaque colony variant were smaller than normal and surrounded by a thick layer of pink-staining material, which he regarded as a mucoid capsule; sometimes two or more vibrios were enclosed in a common matrix. Olsson (1915) described a non-motile variant, derived from atypical wrinkled colonies. The writer has also observed similar non-motile variants in 'type cultures' of *V. choleræ*. Thus non-motile variants may apparently occur associated with changes in colony appearances.

The length of the flagellum is somewhat variable, measuring up to 4 or 5 times the length of the vibrio. Long flagella are frequent but short vibrios with short flagella may be seen. Kolle and Prigge (1927) have figured these two morphological types, namely, short ovoid organisms with short flagella and longer forms with long flagella.

Movement is most marked at 37° C. but becomes less active at lower temperatures and ceases at 16° C.

The demonstration of flagella is referred to later.

Appearances in Dejecta and Tissues.

In dejecta *V. choleræ* shows usually the typical appearance; both short and long forms are noted and sometimes spirals. In films made from the 'rice' flakes of a cholera stool the vibrios may be numerous, and may be arranged with their long axes parallel—often likened to 'fish in a stream'. In tissues the appearance is usually that of a short straight rod-shaped organism, though some degree of curvature may be noted in certain units.

Involution Forms and Morphological Variations.

In old cultures atypical and quite irregular forms occur. A variety of shapes may be observed, e.g. straight organisms, thicker and swollen individuals, spherical forms with faintly stained centres, spindle-, club-, and pear-shaped organisms, individuals with irregular swellings, long swollen spirals measuring up to 17μ, and cells which present a completely distorted structure. Morphological variations have been extensively studied and have been interpreted in various ways. The literature on this subject has been reviewed in detail by Löhnis (1921) who regards such alterations as phases in a supposed life-cycle. Hüppe (1885) claimed that certain of the spherical forms represented resistant 'arthrospores' formed by a process of encystment, but there has been no confirmatory

evidence that these are more resistant than other forms (as indicated originally by Kitasato, 1889). The occurrence of such distorted forms in cultures is hastened by the presence of a fermentable sugar and the formation of acid, by the addition of salt to the medium in a concentration greater than that usually incorporated in media (as in the case of *B. pestis* —q.v.), and by various antagonistic chemical substances. Bittrolff (1912) described very large spherical organisms in media to which various proportions of salt had been added. Gamaléia (1900) noted the rapid occurrence of giant spirilla and globular cells in bouillon containing 1 per cent. lithium chloride. Dowdeswell (1889–90) described branched filaments and large cells of triangular shape. Metchnikoff (1894) found forms with lateral buds and branches. Cunningham (1897) drew special attention to the morphological variations of vibrios under certain circumstances, and stated that these organisms might multiply indefinitely in the form of cocci and short, straight rods. Kohlbrugge (1901) described a ' cladothrix-like ' appearance of *V. choleræ* in a serum medium. In a type culture of a paracholera vibrio after several days' growth in peptone water, the writer has noticed an appearance similar to that described by Kohlbrugge, elongated filaments and chains interlacing and simulating false branching and mycelium formation. Stamm (1914) in a survey of the variations of *V. choleræ* has also described and figured different atypical morphological forms of the organism. Baerthlein (1912 and 1918) has studied mutation of the cholera vibrio and has described three types of colonies with different cell units. Babés (1889) observed an appearance of ' budding ', round bodies occurring at the poles of the vibrio and developing later into typical forms.

V. choleræ is undoubtedly polymorphic in artificial culture, and particularly in old cultures or in media to which certain antagonistic substances have been added. Such pleomorphism is, no doubt, due in part to degeneration of cells and autolytic changes following death of the organism, plasmolysis, changes in the hydrogen-ion concentration of the medium, &c. Undoubted variants occur in *V. choleræ* cultures such as those described by Baerthlein and others, and atypical units may be characteristic of these. The morphology also varies with the stage of growth of a culture. Wherry (1904) has stated that even in a single culture variation may be seen according to the part of the growth from which the microscopic preparation is made; at the edge of the growth where young forms are actively dividing, the organisms tend to be short and almost oval in shape as compared with the centre of the growth where older forms have lengthened and begun to involute. Henrici (1925) claims to have seen all the various atypical forms described by previous writers, at some stage of the growth of a single culture. He suggests that the organism goes through regular metamorphoses from the forms in the original inoculum to the typical vibrio, succeeded by spherical individuals and forms with lateral or terminal ' buds ': after growth starts, the organisms in the inoculum are replaced by thick,

straight bacillary forms, later by more slender curved cells representing the typical vibrio; these persist for 36 hours and then atypical forms as in the original inoculum return, e.g. irregular shapes, spherical cells (varying in size up to 'giant coccoids') long spirals, &c. He states that the typical vibrio appears when growth is slowed and persists till growth stops. He recognized, therefore, three morphological categories: (1) a bacillary 'embryonic' form characteristic of active growth, (2) the typical vibrio which he regards as a resting phase, (3) the irregular forms (spiral, coccoid, &c.)—a senescent phase. He points out also that 'bud-forms' regarded by other writers as reproductive are probably dead structures. Henrici concludes that the apparent morphological changes reported in the literature depend on variations in the rate of growth on different media or of different strains on the same media.

There seems no reason to believe that the atypical forms of *V. choleræ* are other than involution appearances. The fact that they occur in cultures of some duration after growth has stopped and when many of the individual organisms are dying and autolysing supports this explanation. Further, the various irregular forms described are such as might reasonably be expected to result from cell degeneration and particularly autolysis following death. It must be recognized, however, that certain morphological variations may be associated with other biological variations of the organism, e.g. in colony characters, antigenic constitution, &c. (vide Balteanu, 1926).

Staining.

V. choleræ stains readily with all the solutions of basic aniline dyes used in bacteriological work, but generally less strongly than various other bacteria. For simple staining, 1 to 2 minutes application of a 1 in 5 dilution of Ziehl's carbol-fuchsine yields satisfactory results. Shorter exposures to weaker solutions, e.g. 1 in 10, stain the organism rather faintly. *V. choleræ* is definitely and uniformly Gram-negative. In tissue sections, it can be demonstrated by staining with Loeffler's methylene blue and differentiating with acetic-acid-alcohol. For the demonstration of flagella, Loeffler's and Zettnow's methods have been used by many workers. The method of Nicolle and Morax is convenient and reliable. Most uniform and satisfactory results are obtained by the recently described technique of Kirkpatrick (1927).

Cultivation of Vibrio choleræ and its Cultural Characters.

By T. J. Mackie.

Conditions necessary for Artificial Cultivation.

Reaction of medium. *Vibrio choleræ* is an easily cultivated organism, and growths can be obtained on all the ordinary nutrient media in general use. It has been long recognized, however, that even slight acidity of the medium restrains growth and that the organism flourishes on a highly

alkaline substrate. The influence of alkali on the growth of the cholera vibrio was alluded to in 1893 by Hesse, and the capacity of the organism to grow in media of such alkalinity as to inhibit other intestinal bacteria has been extensively utilized in the isolation of pure cultures from dejecta. It may be noted that this tolerance to alkali is also possessed by many other vibrios and is not a special character of *V. choleræ*.

In peptone water of varying initial pH, growth is observed after 24 hours within the range pH 5·8 to 10·6 ; after 72 hours growth is also noted in the same medium with an initial pH up to 11·4 ; the optimum is about 8·2*. Iyengar (1920), in testing comparatively the growth of *V. choleræ* in a tryptic-digest bouillon standardized to varying reactions from −15 (approximately pH 10·8) to +15 according to Eyre's scale, found that between the neutral point (approximately pH 8·2) and +15 there was definite inhibition, increasing with the degree of acidity. In diagnostic work in which peptone water is used for the enrichment of *V. choleræ* in primary culture from dejecta, experience has shown that the best results are obtained by setting the medium to an initial pH of 8·0 to 9·0 (see *Medical Research Council, Special Report,* No. 51).

Further reference, under ' Selective Media ', will be made later to the remarkable tolerance of this organism for alkali.

Aerobiosis. V. choleræ is an aerobe as Koch first showed. It has been generally stated that the organism is a facultative anaerobe, and that slight growth occurs in the absence of free oxygen. In tubes of fluid medium the organism grows most luxuriantly at the surface and forms a surface pellicle ; this has been generally regarded as significant of the aerobic tendencies of the organism, but depends also on other factors (vide p. 353). In solid media, however, inoculated by the stab method, growth occurs along the line of inoculation even in the depth of the medium. Thus the organism shows a capacity to grow under anaerobic conditions in spite of its preference for the aerobic state. Hesse (1893) stated that the complete exclusion of oxygen entirely prevented growth though even a minimal trace allowed the organism to develop. If plates are inoculated and incubated under strict anaerobic conditions in a McIntosh and Fildes jar, the anaerobiosis being controlled by a methylene-blue indicator (see Fildes and McIntosh, 1921), growth results, though it is less abundant than that under aerobic conditions.

According to Hirsch (1926) growth occurs freely under anaerobic conditions in the presence of fermentable sugars ; in this way he explains the capacity of *V. choleræ* to flourish abundantly in the bowel.

Temperature range and optimum temperature. Growth occurs over a wide temperature range ; it is abundant from 30 to 40° C., and the optimum temperature is about 37° C. Growth results even at room temperature, though more slowly than at the higher levels. Under 16° C. it is practically arrested (Koch), though slow development may proceed even at 8° C.

* Observations by M. H. Finkelstein, B.Sc., Bact. Dept., Edin. Univ.

Cultural Characters.

Colonies on nutrient agar (pH 8·0). The colony appearances are subject to some degree of variation, which will be discussed later. A typical and characteristic form of colony can, however, be recognized. Such colonies appear after about 24 hours' incubation at 37° C. as small, translucent, moist, circular discs of growth about 1 to 2 mm. in diameter. Their development continues progressively, and they may attain a diameter of 5 or even 7 mm. after several days. In mixed cultures from dejecta, they show a marked contrast with colonies of *B. coli* in respect of their well-defined, uniform, circular contour and their much greater transparency. If examined under the low power of the microscope they are seen to be almost perfectly circular and regular in outline ; their centres appear more dense and granular than the periphery. Young colonies are practically colourless or greyish-white, but older colonies assume a yellowish-brown coloration.

A stroke growth on an agar slope after 24 hours' incubation consists of an abundant, moist, semi-transparent, confluent layer which is greyish-white in colour ; on continued incubation it becomes more raised and assumes a greyish-yellow tint which deepens after about 10 days to a brown colour.

Colonies on gelatin. On gelatin (incubated at 22° C.), the colonies are specially characteristic. After 24 hours they may be noticed even with the naked eye as small clear points of growth ; as they develop further they appear as exceedingly transparent circular discs, which on magnification, present a somewhat granular surface. The difference between these transparent colonies and the opaque colonies of *B. coli* is even more striking than the respective growths on agar. Young colonies show no colour, but older growths may assume a yellowish tint. After 2 or 3 days, liquefaction of the medium begins under the colonies, which appear to sink into the medium, and, when examined with the microscope, show a central granular area of yellowish colour and a clear border. The outer contour, which is sharply defined, is formed by the edge of the cup of liquefaction, and the central portion of the colony consists of a mass of growth which has settled in the central part of the cup, and has become irregular and broken up at its margin. Liquefaction continues progressively, until the whole medium after about 10 days becomes liquefied.

Gelatin stab culture (at 22° C.). Along the needle track a thin white line of growth occurs, but development is most marked at the surface. In 2 or 3 days liquefaction starts at the surface and a cone or funnel slowly forms. At the top of the funnel evaporation takes place, so that by the fourth or fifth day there is the appearance of an air bell superimposed on the liquefied medium. Liquefaction is progressive, and after about a fortnight the whole gelatin in the tube becomes fluid. Great stress has been laid on this characteristic liquefaction of gelatin by *V. choleræ*, as contrasted with certain other vibrios which produce a different form of

growth in gelatin stab cultures, e.g. *V. proteus* (see p. 432). Liquefaction is due to a proteolytic enzyme; this product of the organism has been demonstrated in culture-filtrates (Baujean, 1913).

Growth on coagulated blood-serum. Abundant growth results at 37° C., similar to that on nutrient agar. Even after 24 hours, commencing liquefaction of the medium is noted, and after several days the whole medium contained in the tube becomes fluid. This property of liquefying solid serum is related to that of gelatin liquefaction, being likewise significant of the proteolytic activities of the organism. The power of liquefying gelatin and coagulated serum is subject to some variability. Long cultivated strains may show partial or complete loss of this property, liquefaction being slow or absent. Even among freshly isolated strains there is some variation in the rapidity of liquefaction.

In fluid media such as *bouillon* or *peptone* water, after 12 to 24 hours' incubation, the growth forms a uniform turbidity throughout the fluid with a thin semi-transparent fragile pellicle on the surface. The pellicle becomes thicker and more coherent and may after several days' incubation sink in the medium. Pellicle formation has been interpreted generally as a function of the aerobic character of the organism, but depends to a considerable extent on the alkalinity and nutritive properties of the medium, and varies with different strains. It was shown by Iyengar (1920) that in acid media (in which growth is restrained) the pellicle is absent. Certain variant types of *V. choleræ* may produce a very thin coherent pellicle with practically no growth in the substance of the medium (vide p. 354). Wherry (1904) likened pellicle formation to that observed in the case of *C. diphtheriæ* and certain other organisms, and found that the property of pellicle formation could be established by serial transfers from the surface growth, i.e. by a process of artificial selection. Beauverie (1916) has stated that in bouillon the addition of 3 per cent. sodium chloride increases the surface growth. The growth in peptone water (pH 8·0 to 9·0) is rapid, especially at the surface, being marked even after 6 hours' incubation at 37° C. Thus, in mixed cultures with other intestinal bacteria, *V. choleræ* may outgrow these and in the surface pellicle may occur in almost pure culture. This property allows of rapid enrichment of the organism in cultures from the intestinal dejecta (vide *infra*).

On *potato slopes* (alkalinized by steaming in 0·7 per cent. sodium carbonate solution) a fairly abundant growth is usually obtained after incubation at 37° C. On this medium chromogenesis is frequently marked, the final coloration being yellow, greyish-yellow, yellowish-brown or pink. An alkaline reaction is essential for growth on potato. Such growths show considerable variations in degree and in pigmentation, which depend both on the particular strain, and also apparently on the specimen of potato used. At room temperature growth is practically absent.

In *milk*, *V. choleræ* grows well but without at first much apparent change in the medium. Slow peptonization occurs. Most strains have no fermentative action on milk-sugar, but coagulation of milk has been

recorded by certain observers. Wherry (1904) emphasized the property of fermenting lactose and described strains which produced acid in a milk medium (without coagulation); V. and A. Bourovie (1912) recorded coagulation of milk as a character of strains isolated during the Russian epidemics of 1908–10; Popoff–Tcherkasky (1914) found among strains isolated during the Balkan War that the majority coagulated milk; the same property has been referred to by Kendall, Day and Walker (1914) who regarded the effect as due to acid fermentation of lactose. This will be referred to later under biochemical reactions.

Variations in Cultural Characters.

As originally described by Kolle and Gotschlich (1903) colonies may show variations in type, some being highly transparent, others yellowish and more opaque; and strains may differ according to the predominance of one of these types. In 1908 Berestneff recorded the occurrence in cultures of *V. choleræ* and certain allied vibrios, of variants producing rough, dry, colonies which in saline suspension underwent spontaneous agglutination. Such variants would appear analogous to the 'rough' variants described later by Arkwright (1920, 1921) in the Salmonella group.

The whole subject of biological variation of *V. choleræ* has now been extensively studied. Morphological variation has already been referred to in a previous section. In 1912, Baerthlein described three forms of the organism represented by colonies showing different characters on culture medium. At that time he recognized the following different colony types: a normal transparent colony of bluish tint; a yellowish-white opaque colony like that of *B. coli*; and a 'ring' colony with an opaque centre and transparent border. Similar variations were also noted by Eisenberg (1912). Later, in 1918, Baerthlein described 10 different colony variants some of which he regarded as subvariants. Certain of these variants were unstable, others proved stable. Shousha (1924) described a 'rough' form apparently analogous to that described by Berestneff. Balteanu (1926) has made a further study of this subject and has described the following variants from the normal circular translucent colonies: (1) *Circumvallate rugose colonies:* small, opaque, yellow with thickened border and radial rugæ or ridges, firmly adherent to the medium. These colonies could not be emulsified in saline. Subcultures in fluid medium consisted of a thick wrinkled film. This variant proved unstable and in subcultures tended to revert to the normal type; (2) *White ring colonies:* white and semi-transparent, sometimes with an opaque centre and transparent border similar to the ring colonies described by Baerthlein. This type also tended to revert to the normal on subculture; (3) *Opaque:* round, very prominent, opaque colonies with an irregular surface; dense, white, firm and adherent, and difficult to emulsify. In fluid medium a thick tough pellicle was formed, the medium remaining clear. The units of this type were found to be non-motile (vide p. 348). This type of colony was

apparently similar to the opaque variant of Baerthlein. Gelatin liquefaction occurred slowly. In subcultures on solid media this variant remained stable, but in fluid media reverted to the normal form.

The serological properties of these variants were also studied by Balteanu, and are dealt with in a later section (p. 369).

Gildemeister (1922) has drawn attention to the occurrence of ' dwarf-colonies ' (' Zwergkolonien ') of the cholera vibrio and other vibrios. Such colonies after 24 hours may not exceed in size those of a streptococcus.

Thus considerable deviation from the characteristic form of colony, associated with variation in other characters, may be encountered. These do not, however, represent stable mutants, but are to be regarded as fluctuating variations of the organism. Such variations are more likely to occur in artificial cultures after continued growth on medium, but may be met with even in newly isolated strains.

Selective Media.

Selective Media for Enrichment.

Reference has been made to the enrichment of *V. choleræ* in *alkaline peptone water*. This constitutes a cardinal method for the isolation of the organism from dejecta, &c., and its practical value has been fully established through long experience. In this medium *V. choleræ* grows rapidly, particularly at the surface, even outgrowing coliform bacilli present in the inoculum, and often within 6 to 8 hours a surface pellicle of growth forms, in which *V. choleræ* is present in almost pure culture. The medium is essentially simple, consisting of 1 per cent. of a suitable peptone and 0·5 per cent. sodium chloride in water, and is standardized to pH 8·0 to 9·0. In making cultures from dejecta, tubes containing about 10 c.cm. of the medium are used.

An *alkaline-egg-peptone medium* has been advocated by Goldberger (1913) for selective enrichment. This medium has been recommended as an alternative to alkaline peptone water in the Medical Research Council Special Report (1920) on the ' laboratory diagnosis of intestinal infections'. It is composed of whole egg mixed with an equal volume of water, to which mixture is added an equal volume of 5 per cent. sodium carbonate ; the alkaline-egg preparation is finally mixed with nine parts of peptone water. This medium has the advantage that *V. choleræ* continues to multiply in it for a longer period than in peptone water. Though the vibrio maintains itself better, it does not increase so rapidly.

Ottolenghi (1911) recommended an *alkaline bile* medium for enrichment. It consists of ox bile to which is added 3 per cent. of a 10 per cent. solution of sodium carbonate and 0·1 per cent. potassium nitrate. The advantages claimed are that other intestinal bacteria grow sparsely, that within a few hours *V. choleræ* is enriched in the medium, and a large amount of fæces can be used for inoculation without interfering with the enrichment. This medium has been extensively tested by various workers ; Bocchia (1911), Weisskopf (1911), and Haendel and Baerthlein (1912) found that

some strains of the cholera vibrio seemed to grow better in peptone water than in the bile medium ; on the other hand, as Haendel and Baerthlein showed, certain strains enrich better in Ottolenghi's solution than in water. They suggested that the two media might be used together, the peptone one being complementary to the other. Krombholz and Kulka (1912), after a detailed study of Ottolenghi's medium, reported that it was not so favourable to the growth of *V. choleræ* as peptone water.

Kraus, Zeki Zia and Zubrzciky (1911) advocated a *blood-alkali-bouillon* prepared by adding 25 c.cm. of the blood-alkali mixture of Dieudonné (vide *infra*) to 100 c.cm. neutral bouillon. They emphasized the marked effect of this medium in suppressing other fæcal bacteria and enriching *V. choleræ*, but it was found that it sometimes also inhibited the cholera vibrio unless each batch of blood-alkali was tested beforehand to determine the optimum proportional mixture of this ingredient with the bouillon. Thus the proportions might require to be varied. Haendel and Baerthlein (1912) state that this medium yields better results than peptone water, but agree with Kraus and his co-workers that a preliminary test of each batch of blood-alkali is required. Dieudonné (1909), Neufeld and Woithe (1910), Pergola (1910) and Hachla and Holobut (1909) also investigated the enriching effect of blood-alkali incorporated in fluid media, but elicited no superiority of such media over alkaline peptone water.

Selective Media for Plating.

In 1909 Dieudonné introduced an alkaline blood agar as a selective medium for the direct isolation of *V. choleræ*, and this preparation has probably been more used than any other medium for isolating pure cultures of the organism. It consists of a neutral nutrient agar to which is added a blood-alkali mixture in the proportion of seven parts of the former to three parts of the latter. The blood-alkali preparation is obtained by mixing equal volumes of ox blood and a normal solution of sodium hydrate and heating the mixture at 100° C. The main difficulty in the use of this medium, but not an insuperable one, is that the blood-alkali mixture must be freed from volatile ammonia or ammoniacal products before it is suitable for use. The original method recommended for the purpose was to prepare plates of the completed medium and allow them to stand at room temperature for 24 hours before use. The writer has found that by repeated steaming of the blood-alkali until the ammoniacal odour is removed, the completed medium prepared from it can be used immediately and the blood-alkali can be kept for considerable periods without losing its selective properties. The removal of ammonia can be tested by preparing plates of the complete medium and inoculating with a known *V. choleræ*. Hall (1916) has recommended keeping the mixture in flasks with cotton-wool stoppers for 6 to 8 weeks, when it can be used for immediate cultivation of the vibrio. The medium is opaque, but this does not constitute a serious disadvantage. It has been pointed out that Dieudonné's medium is inconvenient for application in field work owing

to the difficulty of obtaining blood under such conditions, but it should be noted that the blood-alkali preparation is quite stable and retains its properties for a considerable period, so that it can be made up in central laboratories for use later in the field. The blood-alkali can be prepared in powder form for use in field work (Fürst, 1916). The complete medium has also been made up in the form of a powder which is dissolved in water, heated at 100° C., and then used for pouring plates (see Haendel and Baerthlein, 1912). Dieudonné's medium is highly selective. *V. choleræ* and other vibrios grow abundantly on it, while the usual types of *B. coli* are completely suppressed. Thus, by direct plating from dejecta a pure culture of *V. choleræ* may be obtained. Certain intestinal organisms are found, however, to grow on the medium, e.g. *B. fæcalis alkaligenes*, some unusual types of *B. coli*, certain types of intestinal cocci, *B. pyocyaneus* and *B. proteus* (see Glaser and Hachla, 1911). It is noteworthy, as was shown by Bürgers (1910) and by Haendel and Baerthlein (1912), that growth on this medium does not influence the agglutinability of the organism.

Certain workers have recommended the addition of crystal violet to Dieudonné's medium with a view to intensifying the selective action (Hofer and Hovorka, 1913 ; Fügner, 1914). For this purpose, 0·5 c.cm. of a 1 per cent. solution in water has been added to 10 c.cm. of the medium.

Neufeld and Woithe (1910) in order to obviate any delay in using plates of Dieudonné's medium added 2 c.cm. of 10 per cent. lactic acid to 100 c.cm. of the medium, but it was found that this preparation soon lost its selective property.

Pilon (1911) substituted 12 per cent. sodium carbonate for sodium hydrate in the Dieudonné medium, and found that plates could be inoculated 45 minutes after pouring.

Esch (1910) advocated the used of an alkaline-hæmoglobin agar prepared by dissolving 5 gm. of horse hæmoglobin (Merck) in 30 c.cm. half-normal sodium hydrate which was then mixed with a nutrient agar (15 c.cm. to 85 c.cm.). The plates of medium could be used after an hour. Friedrichs (1911) emphasized the value of Esch's medium, and stated that it was better than Dieudonné's. Esch also described an alkaline medium in which the blood-alkali was replaced by a meat-alkali preparation (500 gm. beef dissolved in 250 c.cm. normal sodium hydrate), but this presented the same difficulty as the blood-alkali medium, and plates could not be used for 24 hours. It was, however, applicable where blood could not be obtained.

Kabéshima (1913) has also modified Dieudonné's medium by replacing blood with a hæmoglobin extract, and Baerthlein and Gildemeister (1915) have reported favourably regarding this medium.

Crendiropoulo and Panayotatou (1910) recommended an alkaline peptone agar, and stated that it restrained the growth of *B. pyocyaneus*. The medium is transparent and can be used immediately after the plates

are prepared. It is made up of six parts of peptone agar with four parts of alkaline peptone, 200 c.cm. of which contain 8 c.cm. of a 10 per cent. solution of sodium hydrate.

In 1912 Haendel and Baerthlein made a careful comparison of the selective plating media in use up to that time and reported that Dieudonné's was the most satisfactory; they found Neufeld and Woithe's modification highly selective but the growth of *V. choleræ* was sometimes restrained.

Krumwiede, Pratt and Grund (1913) recommended alkaline-egg as a substitute for blood-alkali in the Dieudonné medium. The preparation was obtained by mixing whole egg with an equal volume of water and then adding to this mixture an equal volume of 12 per cent. sodium carbonate. They stated that the completed medium in plates could be used after 30 minutes.

Goldberger (1913) in a critical study of various selective media, found that Crendiropoulo's medium was not sufficiently selective, and that Krumwiede, Pratt and Grund's medium was inhibitory also to *V. choleræ*. Goldberger himself advocated an alkaline-egg-glucose agar, and stated that as compared with Dieudonné's medium it yielded a more luxuriant growth with more distinctive colonies, was more suppressive of other bacteria, and that the ingredients were easily obtainable and the plates could be used at once. The medium consists of one volume of an alkaline-egg solution with five volumes of glucose-agar. The alkaline-egg is made by mixing whole egg with an equal volume of water and adding an equal volume of 6·5 per cent. sodium hydrate.

Aronson (1915) introduced a differential selective medium for *V. choleræ*, consisting of 100 c.cm. peptone agar to which were added 6 c.cm. of a 10 per cent. sodium carbonate solution, 1 per cent. saccharose, 1 per cent. dextrose and the fuchsine-sodium-sulphite indicator used in Endo's medium (for the isolation of *B. typhosus*, &c.). On this medium, *V. choleræ* grows well and produces red colonies. Bötticher (1915) stated that it was not so satisfactory as Dieudonné's medium. Baumgarten and Langer-Zuckerkandl (1917) found that the addition of hæmin increased the sensitiveness of the medium.

Among the various intestinal bacteria, *V. choleræ* is unique in its fermentative action on starch, and Gibson (1916) has recommended as a differential medium an alkaline agar medium containing potato starch and litmus (as an indicator). On this medium, the colonies of *V. choleræ* are recognizable by their pink coloration even after 18 hours' growth. Lange (1916) advocated an alkaline agar medium containing rice starch. Kodama (1922) has also applied an alkaline agar medium containing potato starch, ox serum and fuchsine-sodium-sulphite (the indicator of acidity used in Aronson's medium). On this medium, *V. choleræ* in virtue of its diastatic action produces distinctive red colonies.

Teague and Travis (1916) advocated a differential agar medium prepared from a sugar-free meat infusion and containing nutrose and 1 per cent.

saccharose, to which are added eosin and Bismarck-brown. On this medium *V. choleræ* colonies are relatively large and show red centres while those of *B. coli* are entirely pink.

Isolation of Pure Cultures of *V. choleræ*.

From a Patient during Life or Cadaver Post Mortem.

A fresh specimen of the dejecta is obtained or in fatal cases the contents of the small intestine *post mortem*. It is of value to ascertain before making cultures the relative numbers of vibrios present. For this purpose the characteristic white flakes are examined microscopically. Several of these are picked out with a platinum loop and broken up on microscopic slides ; the films are then dried and stained with dilute carbol-fuchsine. If vibrios are numerous, flakes are picked out of the specimen, washed with several changes of sterile saline solution, and then used to inoculate plates of Dieudonné's medium by the successive stroke method as in plating stools from cases of enterica. Flakes are also washed and introduced into tubes of the alkaline peptone water medium described on p. 355. If the dejecta consist of ordinary fluid fæcal matter, a large loopful of the stool is added to a tube of peptone water. The Dieudonné plate is examined after 12 to 18 hours' incubation. If colonies have developed, microscopic preparations are made from three or four of them and stained by dilute carbol-fuchsine. If vibrios are recognized, subcultures from single colonies of the organism are made on agar slopes. These would constitute pure growths for further study.

The peptone water tubes are incubated for 6 to 8 hours. A loopful of the surface growth is then removed by means of a platinum wire with a terminal loop bent at right angles to the wire ; from this, a film is made (without spreading it) on a microscope slide, slowly dried and fixed by heat. The preparation is washed in a stream of water to remove the dried peptone particles, which tend to stain deeply and obscure the organisms. The film is then treated for one minute with dilute carbol-fuchsine. A hanging-drop preparation from the surface of the culture may also be examined ; at the edge of the drop vibrios may, if present, be easily detected by their morphology and motility. Generally the stained film can be relied on. If vibrios are present a subinoculation is made on a Dieudonné plate, which is then incubated, and, if colonies develop, subcultures are made on agar slopes.

If the peptone water culture shows no vibrios or only a few, a subinoculation should be made from it in a second tube, which is also incubated and examined (as before) after 6 to 8 hours. Krumwiede (1911) pointed out that after 8 to 12 hours the first peptone water culture usually contains a practically pure growth of *V. choleræ* at the surface, but that in isolating the organism from carriers and mild cases a subinoculation in a second tube may be necessary to bring about the necessary enrichment, the first tube showing few or no vibrios. If vibrios are not detectable in the first tube or if they are scanty as compared with other bacteria, further

enrichment may sometimes occur in the second culture. If vibrios are numerous in the stool they usually produce an abundant growth on the direct Dieudonné plate, and this growth as a rule is practically pure, so that for purposes of immediate identification an emulsion for the agglutination test can be prepared from it.

Isolation from Carriers.

Methods have been suggested for the isolation of the cholera vibrio from the gall-bladder (where they are resident) : (1) by giving large doses of olive oil, followed by siphonage of the stomach contents, in which regurgitated bile is found ; (2) by introducing an Einhorn tube into the duodenum. The material obtained would be cultured as in dealing with dejecta.

For general routine work, however, the fæces are examined after mild purgation ; one or two loopfuls of the fluid portion of the stool is introduced into peptone water, the procedure for enrichment being the same as that described above in the case of choleraic patients. It is pointed out in the Medical Research Council Special Report (1920), that by enrichment in peptone water *V. choleræ* can be isolated when only 4 to 8 vibrios are present in 25 c.cm. of a dense fæcal emulsion.

Isolation from Water.

To 900 c.cm. of the water specimens are added 100 c.cm. of a sterile alkaline peptone solution containing 10 per cent. peptone and 5 per cent. sodium chloride. The mixture is divided up and distributed in a number of conical sterile stoppered flasks. These are incubated and the surface growths are examined for vibrios and subinoculated on Dieudonné plates (as in the cultivation of *V. choleræ* from fæces) after 12 hours, and also after 24 or 48 hours if previous examinations have been negative in result. Pure cultures are then obtained from isolated colonies.

Viability and Maintenance of Cultures.

V. choleræ, when adapted to growth on culture medium, remains viable for considerable periods. On agar slopes, if kept at room temperature in the dark, and if drying is prevented by covering the mouth of the tube with a rubber cap or a paraffin seal, it survives for 2 or 3 months. To maintain stock cultures, agar slope growths kept as described above are subcultured at intervals of one month. Recently isolated cultures should be subcultured more frequently.

Bacteriophage Effects (Transmissible Lysis).

Transmissible lysis of the cholera vibrio has been observed and studied by several workers. D'Herelle (1926) raised the question as to whether the apparent bactericidal action of certain river waters in India, originally described in 1896 by Hankin, was due to bacteriophage action. Hankin, in laboratory experiments with the filtered water of the Jumna artificially contaminated with *V. choleræ*, found that after a few hours the vibrios

had been completely killed, while the boiled water was inactive in this respect, and even allowed the organisms to increase. At that time no definite interpretation was made of the phenomenon, and it has not been subsequently studied or confirmed.

D'Herelle (1926), in his earlier work on the cholera bacteriophage, was generally unsuccessful in demonstrating a lysin for *V. choleræ* in fatal cases, but, in one convalescent, found that a filtrate of the stool produced in a plate culture of *V. choleræ* the plaques characteristic of phage action, though it was not lytic towards a suspension of the organism. He was not able, however, to ' cultivate ' the phage in series. D'Herelle stated that he had isolated a *V. choleræ* bacteriophage by combining *in vitro* a mixture of the peritoneal fluid of a guinea-pig (after intraperitoneal injection with bouillon) and an anticholera serum together with a culture of the organism.

Jötten (1922) claimed to have demonstrated bacteriophage in an old culture. Meissner (1924) isolated a bacteriophage active towards *V. choleræ* and also the El Tor vibrio, from the peritoneal fluid of a guinea-pig which had been injected with *V. choleræ*, the El Tor vibrio and anticholera serum. He found its activity increased by serial transfers. Flu (1924) has also recorded the isolation of a *V. choleræ* phage from a pure culture originally obtained from a case in 1915. It was only active, however, for 1 out of 10 strains tested and was inactive for the strain from which it was isolated. He found it highly sensitive and he could not ' cultivate ' it along with a lysogenic strain. For its ' cultivation ' and preservation it was necessary to inoculate broth with strains which were readily lysible and to use a very small amount of a young culture. As soon as lysis was complete, after a few hours' incubation, the medium was filtered and a new inoculation made. This phage preparation was found to be lytic in a dilution of 1 in 10^7. D'Herelle tested Flu's phage and found its action restricted to a minority of *V. choleræ* strains.

V. choleræ bacteriophage has been studied recently by Nobechi (1926), who found that among 18 strains 3 were spontaneously lysogenic, and filtrates contained typical bacteriophage. Strains were found to vary in their susceptibility to the lysin. He also noted that cultures modified by bacteriophage became inagglutinable when kept without subculture.

Ciuca (1923) found in the stools of cholera cases a transmissible lysin active for *B. dysenteriæ* Shiga and *B. coli*, but not for *V. choleræ*. He suggested that the intestinal contents in cholera, if rich in leucocytes, are favourable to the development of a lytic principle active towards *B. coli* and *B. dysenteriæ* Shiga, though there is no similar development in the case of *V. choleræ*. It is of special interest in regard to the general question of bacteriophage action or transmissible lysis that lysins for other organisms should have been demonstrated in cholera cases.

D'Herelle and Malone (1927) have recently reported the results of a further study of the occurrence of *V. choleræ* bacteriophage and its significance. They have attempted to correlate the development of bacteriophage with recovery from the infection, and have contrasted

fatal cases in which phage was absent with recovered cases in which a strongly lytic principle could be demonstrated. They also suggest that the subsidence of an epidemic of cholera is related to the generalized excretion of phage from convalescent patients and its distribution by water and flies. Success has been claimed in arresting village outbreaks by addition of phage preparations to wells supplying these villages with water. The observations seem too limited at present to justify any definite conclusions.

Biochemical Properties.

By T. J. Mackie.

Proteolytic Effects.

Reference has been made to the marked proteolytic action of *V. choleræ* (as exemplified by the liquefaction of gelatin and coagulated serum), and the demonstration of protease in culture-filtrates (Baujean, 1913). Such proteolytic effects, while well marked in recently isolated cultures, are to some extent variable and may be completely or partially lost. It must be noted that for the maximum proteolysis to occur, the organism requires an alkaline substrate ; the effect is analogous to trypsin action, and is inhibited by any initial acidity of the medium, or by development of an acid reaction such as might result from the simultaneous fermentation of carbohydrates present in the medium. These factors must be considered, as Wherry (1904) has pointed out, in assessing the significance of both the degree and type of liquefaction of gelatin (vide p. 352), on which stress has been laid as a biological feature of *V. choleræ*. Further, Wherry has shown that the type of liquefaction of gelatin depends also on the melting-point of the medium.

Indole formation. *V. choleræ* produces indole rapidly in a suitable medium, e.g. alkaline peptone water ; this can be demonstrated by means of Ehrlich's rosindole test as applied in the detection of this product in cultures of *B. coli* and other indole-forming organisms.

Cholera-red reaction. This colour reaction, which can be readily elicited with cultures in peptone water, was first demonstrated and studied by Poehl (1886), Dunham (1887) and by Bujwid (1888). If a few drops of concentrated chemically pure sulphuric or hydrochloric acid are added to a peptone water culture a pink or purple colour immediately develops in the medium. In young cultures after 8 to 12 hours' growth, the resulting coloration is pink ; in older cultures after 2 to 3 days' incubation, the tint is purplish-pink, similar to that of a potassium permanganate solution. This coloration, as demonstrated by Brieger (1887), Salkowski (1887) and Petri (1889), is due to the formation of ' nitroso-indole ', resulting from the interaction of indole and nitrites in the medium on the addition of a mineral acid which liberates nitrous acid from its compounds. The presence of nitrite is a function of the reducing properties of the organism acting on the nitrates derived from the meat

extract or peptone present in the medium. Though the reaction occurs in cultures of *V. choleræ* with great constancy, certain factors may interfere with it. Some peptone preparations are unsuitable for the purpose due apparently to lack of tryptophane, from which indole is formed. Tobey (1908) and Logie (1913) have also shown how nitrite-destroying organisms, e.g., *B.coli*, grown along with *V. choleræ* may prevent the appearance of the reaction. This introduces a possible fallacy if the test is applied to mixed cultures from stools. Variations in the nitrate-content of the medium may influence the reaction, and, as Bleisch (1893) first emphasized, greater constancy is attained if nitrate is added to the medium in the course of its preparation (0·01 per cent. sodium nitrate, i.e. 1 c.cm. of a 10 per cent. solution per litre of medium).

This reaction is common to *V. choleræ*, certain non-cholera vibrios and various other organisms and is in no way a specific effect of the cholera vibrio ; on the other hand, certain vibrios are characterized by its absence (e.g. *V. proteus*).

The reducing properties of *V. choleræ* are also well illustrated by growing the organism in litmus-bouillon, the litmus becoming rapidly decolorized. Neutral-red incorporated in peptone water media containing sugars is not reduced and constitutes a satisfactory indicator of acid formation (vide *infra*).

Hydrogen sulphide is not formed in culture medium and no blackening of lead-acetate-agar occurs.

Saccharolytic Effects.

Various sugars and other carbohydrate substances are decomposed with the formation of acids, e.g. lactic acid and other fermentation products, as in the case of the sugar-fermenting Gram-negative bacilli, but *without gas production*. The fermentation of certain of these substances is a constant property of *V. choleræ*. On the other hand, saccharolytic reactions have not served to differentiate *V. choleræ* from allied vibrio species. The fermentative reactions of *V. choleræ* and certain other vibrios, have recently been systematically studied by Nobechi (1925). The following carbohydrates are fermented with great constancy : glucose, lævulose, galactose, mannose, maltose, saccharose, starch, dextrin. According to Pergola (1921) arbutin is acted on by *V. choleræ*. Towards other substances, *V. choleræ* shows uniformly no fermentative effect : xylose, dulcitol, isodulcitol, arabinose, adonitol, inulin and inositol.

The vast majority of strains ferment mannitol, but occasionally, recently isolated strains may be found to ferment this substance slowly and older strains may fail to ferment it altogether. Nobechi has noted that fermentation of lactose and glycerol is inconstant and variable.

Though no true *V. choleræ* strains ferment lactose, within 48 hours occasionally late fermentation of this sugar is noted. Reports of different writers vary as to the occurrence of acid-formation in milk. As a rule, little or no change in milk is observed, though a slight acid reaction may

develop after a few days' growth ; some observers, however, have described acid formation and even coagulation (Wherry, 1904 ; V. and A. Bourovie, 1912 ; Kendall, Day and Walker, 1914, and Popoff-Tcherkasky, 1914). Wherry has claimed that *V. choleræ* produces lactase and invertase and described the formation of acid in milk as a distinct property of certain strains.

The diastatic action towards starch has been referred to on p. 358, in regard to the utilization of this reaction for purposes of differential media. Kodama and Takeda (1922) expressed the view that by this reaction *V. choleræ* might be differentiated from closely allied vibrios, but this has not been supported by others (see Gildemeister and Herzberg, 1923). Dumas (1919) described the hydrolysis of glycogen into glucose, maltose and lactic acid as a constant property of *V. choleræ* and related vibrios.

Hæmolytic Effects.

Hæmolysis by *V. choleræ* growing in a blood-gelatin medium was described by Koch. Eijkman (1901) noted the same effect in cultures on rabbit-blood agar. In 1903, Kraus stated that *V. choleræ* did not produce a filterable hæmolysin and differed in this respect from various other vibrios which produced rapid hæmolysis on blood-agar plates and formed in culture a filterable hæmotoxin.

The question of the hæmolytic action of *V. choleræ* and other vibrios gained particular prominence in 1905, when Gotschlich reported the isolation of six actively hæmolytic strains—the so-called *El Tor vibrio*—which were serologically identical with the true cholera vibrio. They were recovered from the bodies of six Mecca pilgrims who died at El Tor in Arabia as a result of a dysenteric condition, but without cholera symptoms. At that time there was no cholera in Arabia, but the patients had come from countries where cholera was endemic. There can be little doubt now that these patients were cholera-carriers, but the occurrence of carriers was not so fully understood then as in later years. The El Tor strains corresponded in biological characters to the true cholera vibrio, but were strongly and rapidly hæmolytic and contrasted in this respect with *V. choleræ*. They were agglutinated in high titres by an anticholera agglutinating serum and specific antisera prepared from them reciprocally agglutinated *V. choleræ*. They produced an active hæmolysin in culture, and a toxin which was rapidly lethal to experimental animals. As a result of these observations it was concluded by Gotschlich and others that the El Tor strains were not true choleragenic organisms, and the question of hæmolysis as a criterion in differentiating non-cholera vibrios from *V. choleræ* received considerable attention and aroused a certain amount of controversy. The subject has recently been reviewed in detail by Kolle and Prigge (1927).

Kraus and his co-workers emphasized the lack of hæmolytic power on the part of *V. choleræ* as a differential biological feature between it and

allied vibrios (see Kraus and Pribram, 1906 ; Kraus and Prantschoff, 1906 ; Kraus and Fukuhara, 1909 ; Kraus and Müller, 1910 ; Kraus, Graham and Zia, 1911 ; Kraus, 1922). Kraus (1909) designated as ' paracholera ' vibrios hæmolytic strains (such as the El Tor vibrio) which were otherwise identical with *V. choleræ*. It may be noted here that Kraus and his co-workers used sheep and goat blood for their hæmolytic tests, in preference to that from other animals. Schumacher (1906) supported Kraus in his contention that the hæmolytic test was a delicate method of differentiating *V. choleræ* from other vibrios. He recommended calf blood as the most suitable for the test, as being less fragile than that of other animals.

Other writers on this subject have claimed that true cholera vibrios may exhibit undoubted hæmolytic effects. Meinicke (1905) found that a typical *V. choleræ* might produce laking round colonies on blood plates, though culture filtrates contained no hæmotoxin. This occurred both with recently isolated cultures and older strains. Mühlens and v. Raven (1906) and others recorded similar observations. Huntemüller (1911) stated that recently isolated strains of *V. choleræ* were as actively hæmolytic as the El Tor strains, and Kabéshima (1918) reported that of 206 strains isolated 91·6 per cent. showed a hæmolytic effect in solid and fluid media. Hüntemuller and Ornstein (1912) regarded variations in hæmolysis as a quantitative rather than a qualitative difference. They pointed out that after 24 hours' to 6 days' growth a number of *V. choleræ* strains exhibit a hæmolytic effect which is elicited better by using 0·5 per cent. rather than 5 to 10 per cent. of blood in the medium. Inouye and Tatsuo Kakihara (1925) state that the apparent hæmotoxic effect of cholera strains varies with the species of animal from which the blood is obtained ; thus they find horse blood markedly susceptible while sheep blood yields negative results.

Thus the observations and views of different workers have introduced a remarkable discrepancy in regard to the question of the hæmolytic properties of the cholera vibrio. Van Loghem (1913) has suggested that the apparent hæmolysis on blood plates produced by *V. choleræ* is in reality a ' hæmodigestion ', and he has distinguished this effect from true hæmolysis. Thus, by spectroscopic examination he has shown that in the zone of laking resulting from true hæmolysis oxy-hæmoglobin can be observed, while in the case of ' hæmodigestion ' this is absent. Van Loghem emphasized also the contrast between *V. choleræ* and non-cholera vibrios in regard to their rate of producing lysis, *V. choleræ* acting more slowly than other vibrios in this respect. He stated that, to differentiate these organisms by a hæmolytic test, incubation should not be prolonged beyond two hours.

According to Kovács (1926), *V. choleræ* strains which tend to resemble the El Tor vibrio in their lytic effect on blood, also produce a zone of clearing when growing on a coagulated blood agar, while the El Tor vibrio has no effect on this medium. He suggests that *V. choleræ* acts

on the hæmoglobin while the El Tor vibrio affects the corpuscles. Van Loghem (1926) has also pointed out that the hæmolytic agent of *V. choleræ* is an endolysin, in view of the acceleration of its laking action by bacteriophage, whereas that of the El Tor vibrio is an exotoxin.

There is apparently no relation between the hæmolytic and proteolytic action of the organism (Baujean, 1913).

An extensive and careful study of the action of cholera and other vibrios on blood has been made by Greig (1914). This was tested as follows : the strain was grown in alkaline bouillon for 3 days at 37° C., and then varying quantities of the culture—0·01, 0·05, 0·1, 0·5, 1 c.cm.—were added in tubes to 1 c.cm. of a 5 per cent. suspension of goat's red corpuscles in normal saline. The mixtures in the tubes were incubated at 37° C. for 2 hours, and then placed in the ice-chest over-night, readings being made the following day. A number of strains were also plated on 11 to 12 per cent. goat-blood agar, the results being observed after 24, 48 hours, &c. All the true cholera vibrios tested (333 in number) were non-hæmolytic as a result of the test with blood suspensions. In plate culture the great majority of 161 strains tested showed no zone of laking round the colonies, or merely a trace of clearing, within 24 hours. After 24 hours, however, many strains exhibited distinct clearing. Greig was of the opinion, in agreement with van Loghem, that such late effects are probably due not to hæmotoxin, but to other hæmolysing metabolic products. He found also that the great majority of 100 strains of other vibrio species tested, lysed blood suspensions, and some to a pronounced degree. On plates, clearing occurred within 24 hours : this can be interpreted as a true hæmotoxin effect. It is apparent that the test for hæmolysis by means of blood suspensions yields a better contrast between *V. choleræ* and the actively lysing allied vibrios than the blood-plate method.

Van Loghem's distinction between ' hæmodigestion ' and hæmotoxin effects offers a rational explanation of the discrepant observations of different workers which have been referred to above. The occurrence of clearing round growths on blood plates is an undoubted effect produced by the true cholera vibrio, but is distinguished generally from the clearing due to certain other vibrios by its slower development. The absence of lysis in the test with blood suspensions as applied by Greig also indicates that *V. choleræ* has much less power to lake blood than the other types, and it may be said that the typical cholera vibrio produces no characteristic hæmotoxin. On the other hand, hæmotoxin production and rapid hæmolytic action must be regarded as a possible though infrequent character of the species and is well exemplified by the El Tor strains. It has been noted how *V. choleræ* may exhibit fluctuating variations in certain of its biological characters, and, as Kolle and Prigge (1927) have suggested, hæmolytic action or its absence may also be functions of biological variation and not stable characters.

A Specific Chemical Substance of *V. choleræ*.

Landsteiner and Levine (1927) have extracted by hot dilute alcohol a specific chemical substance from *V. choleræ* cultures which they regard as a complex carbohydrate. This specific principle seems to belong to the class of substances originally studied by Avery and Heidelberger (1923) in the case of the pneumococcus. The original alcoholic extract prepared by Landsteiner and Levine contained protein, but from this they separated a protein-free product which was not antigenic *in vivo*, though it reacted specifically with an anticholera precipitin. They regard the specific cholera antigen as a complex of protein *plus* the specific carbohydrate substance on which antigenic specificity depends.

Serological Reactions.

By W. F. Harvey.

The general subject of serology has, to a very large extent, been concerned from the very beginnings of bacteriology with cholera in particular. Thus we find that it is the cholera vibrio or one of its congeners which has been used more frequently than any other pathogenic bacterium as the test organism for all the aspects of serology and immunology. There is no difficulty in obtaining a well-marked antibody or phagocytic response in animals injected with the cholera vibrio. A great deal of recent work has also been done with the cholera vibrio to demonstrate the existence of a localized cellular immunity. The locality of formation of antibodies in cholera is still subject-matter of dispute.

Antibody Reactions.

Of the two main types of antibody reaction, the antibacterial and the antitoxic, it is the antibacterial in cholera which is the more clearly demonstrable. Antibody reactions in cholera, therefore, relate almost entirely to differential serum tests with cholera vibrios.

Agglutination.

The agglutination of the cholera vibrio was not the first of the serum reactions to be discovered or used. The first place is taken in this respect by the bacteriolytic reaction known as Pfeiffer's phenomenon (see p. 370). But agglutination is, in almost all respects, a more important serum reaction than bacteriolysis and may well be said to form the groundwork of the serology of cholera. As a rule the disease itself does not require much more than the clinical diagnosis, although isolation of the vibrio, especially in first cases, may be required for official acceptance of the appearance of cholera. Agglutination and other serum reactions are too slowly developed in patients to afford much help in the identification of the affection.

Specificity.

The rabbit serum prepared from an agglutinable strain of a cholera vibrio obtained from an undoubted case of clinical cholera, occurring in a well-marked epidemic of the disease—and this is what may be designated a 'true' cholera vibrio—will agglutinate the homologous organism in dilutions as high as 1 in 40,000, and will unerringly pick out the same type of cholera vibrio from cholera-like vibrios and other vibrios. Such a serum, on the other hand, affects a cholera-like vibrio in very much the same way as the serum of a normal rabbit. These same cholera-like vibrios are, however, very highly agglutinated by their own high titre sera, and their own sera, similarly, do not agglutinate the true cholera vibrio in much higher degree than a normal serum. The degree of normal serum agglutination varies somewhat in different animals. It is highest in the horse and diminishes in serial order from ox, sheep, dog, rabbit, guinea-pig to man. The titre of normal agglutinins in rabbit serum, which is the serum mostly used in test, does not as a rule exceed 1 in 50 and at the outside 1 in 100. Normal human serum agglutination does not exceed 1 in 10 or 1 in 20. Group agglutination, which may lead to serious difficulty in the typho-dysentery group of organisms, does not cause very much difficulty in the case of cholera. On the other hand, though intermediate degrees do not occur, it is not possible to say that the inagglutinable vibrio is not a cholera vibrio. With cultivation outside the body some of these may become agglutinable. The occurrence of temporarily inagglutinable vibrios is especially observable during times of cholera epidemic, and not only may they be found in stools along with the cholera vibrio, but also in the stools of convalescents, healthy individuals, and the water of wells, village tanks and rivers. The whole subject of agglutinability of the true cholera vibrio, and the statements of older authors must at the present time be revised or modified in the light of the recent applications of serological analysis to various groups of organisms, among them the cholera vibrio (Shousha, 1923–4 ; Balteanu, 1926). In these researches, organisms derived from single colonies have been shown to dissociate into smooth and rough colony forms with the application of various modes of treatment, such as ageing of the culture, growth on phenol or starvation agar, cultivation in immune serum, &c. ; they dissociate into types which, in the case of the rough forms, tend towards loss of virulence, spontaneous agglutinability and altered agglutinability with the commonly used high titre cholera sera. Inagglutinable vibrios will require in future to be tested with special sera.

The cholera vibrio has but one flagellum and it is only to be expected that the flagellar type of flocculation should not be a prominent feature in the reaction. Balteanu (1926) has shown here that, if the flagella be detached from cholera vibrios by shaking, the flagellar suspension will flocculate with cholera serum in loose voluminous form.

Another type of serum agglutination which concerns the cholera vibrio is the pseudo-agglutination of Friedberger and Luerssen (1905), which,

they maintain, is given by 6- to 8-hour-old slope agar cultures from freshly isolated single colonies of the cholera vibrio. The possibility of erroneous findings on this account can obviously be prevented by the systematic use of agar cultures of 18 to 24 hours' growth, by which time any such tendency, if it has existed, will have completely disappeared.

There is a form of agglutination denominated paragglutination which is said to occur with entirely extraneous organisms.

The absorption test of Castellani is an important test, designed to distinguish between group or co-agglutinins and primary or specific agglutinins. In the case of cholera, however, there is not very much evidence of the existence of group vibrios serologically related to the cholera vibrio. The absorption test, however, has its use in confirmation of the simple agglutination test and may have further usefulness in the admittedly difficult case of inagglutinable vibrios, which are yet true cholera vibrios.

Mutability.

Instances of alteration from inagglutinability of vibrios to agglutinability have already been given. The application of a temperature of 100° C. for 2 hours to a suspension of cholera vibrios has little or no effect on the agglutinability of the suspension by cholera serum, that is to say, the agglutinogen or agglutinable substance of the cholera vibrio is highly thermo-stable. It is regarded as being of the 'somatic' type, and contrasts strongly with the thermo-labile 'flagellar' type of agglutinogen. The effect of heat, as shown by Balteanu (1926) on the corresponding antisera is of the opposite description, that is to say, the agglutinin to 'somatic' or thermo-stable agglutinogen is almost completely destroyed by 20 minutes' heating at 70° C., while this degree of heat leaves the agglutinin to flagellar or thermo-labile agglutinogen almost unaltered.

As regards the alteration of agglutinability of cholera vibrios by sojourn in water, its importance lies in this, that vibrios isolated from water sources, especially during times of epidemic cholera, have been regarded as saprophytic forms with potentialities for pathogenic action. These water vibrios are practically always inagglutinable by cholera serum, and unable to act as cholera agglutinogen. Of these characters, the inability to act as agglutinogen, which seems to be a much more persistent character than agglutinability, is a strong, although by no means conclusive, argument against their being true cholera vibrios. Greig (1915[4]) did not find a single one of the water vibrios resembling the cholera vibrios, isolated by him, to be agglutinogenic, nor were any of them agglutinable. Zlatogoroff (1911) for water vibrios, and Horowitz (1911) for vibrios isolated from fæces of convalescents and healthy persons during epidemics, have upheld the doctrine of alterability of the character of *V. choleræ*. Other observers such as Köhlisch (1910) and Haendel and Woithe (1910) have failed to obtain the results of Zlatogoroff. Stamm (1914) found that some of his cholera cultures had their agglutinability removed by passage

through water and others had not, and that these changes were permanent. He insisted, however, on the persistence of agglutinogenic capacity and maintained the value of the agglutination test in the differential diagnosis of cholera vibrios. Tomb and Maitra (1926) have drawn attention to the persistence of non-agglutinating vibrios in the stools of cholera convalescents after the agglutinable cholera vibrio has disappeared. They conclude that this non-agglutinating vibrio, found in the water of village tanks during times of epidemic cholera, is the permanent form in nature of the cholera vibrio and that this is changed into the agglutinating form under favourable conditions in the human intestine.

Correlation of agglutinability with other characters.

The chief characters to be considered are, the serological other than agglutination itself, hæmolytic power and virulence. Bacteriolysis, precipitin reactions, and complement fixation are very highly correlated with agglutination and are for the most part used for confirmation of the results obtained by the agglutination test. Some one or other of these tests has been upheld as divergent or as more specific, but this is not at all well substantiated. It has to be remembered that the Pfeiffer test was the first in the field of serological tests generally, and was the means of advancing our knowledge to a very high degree. There has been naturally, therefore, some reluctance to give up this test as the main test and to adopt the much simpler agglutination test. But there seems little doubt that the two reactions are very highly correlated, and may be looked on as confirmatory of each other rather than as provisional and final tests respectively. The precipitin reaction may be dismissed as fundamentally the same as the agglutination test. Complement fixation has had its advocates as a more delicate means of separating cholera vibrios from cholera-like vibrios than agglutination, but there is very strong evidence that a high correlation subsists between the two characters. As regards hæmolytic power, this raised a controversy commencing many years ago and still continuing. It is the question of the title of the El Tor vibrios, highly hæmolytic and yet agglutinating to titre with cholera serum, to be considered true cholera vibrios. Virulence as a character of the cholera vibrio has not been found to be closely correlated with agglutinability although research with smooth and rough colony forms may serve to demonstrate a connection.

Bacteriolysis.

The bacteriolytic test was introduced by Pfeiffer and is known, in the form in which he applied it, as Pfeiffer's phenomenon. In reality, it represents an *in vivo* mixed agglutination and bacteriolytic phenomenon. It was for a considerable time regarded as essential for the official acceptance of the appearance of cholera, that the first case should have the seal of Pfeiffer's test set upon it. The reaction is now largely superseded by the agglutination test or one of its modifications, but is still employed for the confirmation of the agglutination reaction in difficult sporadic

cases of cholera-like disease. In those cases where the Pfeiffer phenomenon and the agglutination test agree, in a positive sense, in identifying a test vibrio with the cholera vibrio there is very high presumption that the latter is a true *V. choleræ*. But even this position has its contestants and brings up again the subject of the controversy over the title to separate specificity of the El Tor vibrio. The specially selected El Tor vibrios, despatched originally by Gotschlich to Berlin and Vienna, gave the full agglutination and bacteriolytic tests of the cholera vibrio, but differed markedly from it in being highly hæmolytic to the erythrocytes of the sheep and goat, whereas the cholera vibrio is generally credited with possessing no hæmolytic power.

Complement Fixation.

The complement fixation method was used by de Besche and Kon (1909) for the separation of cholera-like vibros from true cholera vibrios and to prove the identity of the El Tor vibrios with *V. choleræ*. They affirmed the serological identity of the El Tor vibrios with, and the serological differentiation of the commonly called cholera-like vibrios from *V. choleræ*. Greig (1915[3]) in his investigations in India found no vibrios representative of the El Tor type, but found that the complement fixation test differentiated cholera-like vibrios from *V. choleræ*. He pointed out also that in such complement fixation researches it is necessary to use a dilute antigen and to place controls on the possibility of destruction of complement by the vibrio antigen itself. Cholera antigen may be more destructive of complement than cholera-like antigen. Mackie (1922), discussing the subject of the paracholera vibrios, obtained results with complement fixation tests which, just as had the agglutination and Pfeiffer's tests, differentiated these vibrios completely from the true cholera vibrio. The real objection to complement fixation as a method of separation of vibrios is that it is complicated as compared with the agglutination test, and appears to possess no sufficient advantage in regard to delicacy to justify its use for this purpose. The method of using stools directly as antigen in complement fixation (Nedrigailoff, 1909) does not, any more than a similar method for agglutination (Dunbar, 1905) appear to have obtained many adherents. It suffices here to mention the fact that cholera stools, though not the filtrates of the stools, can act by fixing complement in the specific test and have been so used with a view to accelerating laboratory diagnosis.

Opsonic Action.

Neufeld and Hüne (1906), in their work on specific phagocytosis by immune sera, definitely used the cholera vibrio as one of their test organisms. They found that a specific phagocytosis was quite on a par with the important specific bacteriolysis of the Pfeiffer test.

Precipitins.

Precipitin reactions are chiefly interesting as linking bacterial antibody reactions with those which take place between protein solutions and their

antisera. The filtrates of old cholera bouillon cultures were shown by Kraus to give precipitates with homologous antisera. There is so close a correspondence between precipitin and agglutinin reactions that there is good reason for taking the reaction to be one and the same.

Anaphylaxis.

A reaction, which is perhaps doubtfully to be included among antibody reactions, and may in fact occur in animals when no demonstration of the presence of antibodies can be given, is the anaphylactic reaction. The whole of the explosive syndrome and algid state in cholera has been referred to anaphylactic shock. It would, according to Sanarelli (1923), be a reaction of the sensitized animal to continued invasion by cholera vibrios and their toxic products ; but there does not seem to be adequate reason to resort to a theory of anaphylaxis when simple toxic action supplies a sufficient explanation.

Serological Differentiation of Species.

The vibrios which are entitled to the grading of species, are organisms which not only show complete serological differentiation from the cholera vibrio, but are differentiated by other characters also. *Vibrio metchnikovi* may be cited as an example, differing especially from *V. choleræ* in the additional character of pathogenicity by intramuscular injection in pigeons and resultant septicæmia.

Serological Races.

It is the serological identity of the much-discussed El Tor vibrios with known cholera vibrios which has led to their being regarded by so many workers as true *V. choleræ*. It is equally the want of serological identity among other vibrios which has led to their being distinguished from true cholera vibrios by such terms as cholera-like vibrios, pseudo-cholera and paracholera. The basis of this serological separation of vibrios is the cross agglutination experiment. The peculiarity of the El Tor vibrios which distinguished them to a very large extent from cholera-like vibrios, was that they reacted with cholera sera just as known cholera vibrios did. They differed markedly from the standard cholera vibrio by their power of developing in culture a hæmolysin to sheep or goat erythrocytes. And thus the question whether El Tor vibrios were true cholera vibrios or not was reduced to one of whether hæmolysis was a sufficiently distinctive character to constitute a specific or racial difference.

There is, however, a class of cholera-like vibrios, markedly differentiated from the cholera vibrio by serological behaviour. Cross agglutination between the cholera vibrio and cholera-like vibrios and between cholera-like vibrios themselves gives evidence of the separation of a series of groups of greater or less size, which are distinctly and clearly differentiated serologically. The process might theoretically go on indefinitely, but comes to an end in any particular investigation by virtue of the conditions

imposed by time and place. The epidemic comes to an end : the material for testing comes to an end : the separation of organisms becomes too rare an event to make continuance of the work worth while, and so on. We are left then with the harvest of groups of cholera and cholera-like vibrios. What seems to emerge from the numerous researches on this subject is that there is sharp serological cleavage between these groups. In the case of cholera there is little or no account in the literature of vibrios which agglutinate only partially with cholera serum. It would seem likewise to be a case of all or nothing with the cholera-like vibrios. They agglutinate or they do not agglutinate with a serum prepared to any one group.

Gotschlich, Hetsch, Kolle, Lentz and Otto (1903) obtained 14 serologically different groups out of 16 cultures of cholera-like vibrios. With a series of 37 water vibrios from wells and tanks, all of which were monoflagellate, motile, produced indole, liquefied gelatin and gave the sugar reactions of the standard cholera vibrio, Greig (1915[4]) obtained, by means of the agglutination and agglutinogenic tests, 6 separate groups, all of them different from the standard cholera group. Groups 1, 2, 3, 4, 5 and 6 contained respectively 10, 3, 5, 4, 5 and 3 individuals and there was a further set of 7 ungrouped individuals.

A very instructive series of cholera-like vibrios actually isolated from stools of clinically undoubted cholera cases at the same time as the cholera vibrio is also given by Greig (1917[2]), which was investigated from this point of view of serological races. The frequency distribution obtained was :

Race	1	2	3	4	5	6	7	8	9	Unclassed	Total
No. of individuals	7	30	4	9	4	3	3	3	2	13	78

Mackie (1922) investigated a series of 57 strains of vibrio from typical cholera cases, from cases of non-choleraic diarrhœa, from convalescents and from healthy carriers, which corresponded in general characters with known cholera strains, but were markedly hæmolytic on blood-agar plates and which neither gave direct agglutination with cholera serum nor cross agglutination tests. Out of the 57 strains 20 serological groups emerged, 8 of which consisted of a single individual only. What seems so extraordinary in these results is that such a very large number of distinct groups were separated out and that many of them contained so few individuals.

Serological Variants.

There is evidence for the statements that in the stools, along with cholera vibrios which agglutinate and otherwise show all the characters laid down for a true cholera vibrio, there may occur others which do not

react serologically while exhibiting all the other characters. The questions arise then : first, whether these non-reacting vibrios are cholera vibrios ; and secondly, if they are, why they should be inagglutinable. There is a greater tendency to find inagglutinable vibrios or less agglutinable vibrios during convalescence than when symptoms are still at their height. Whether such vibrios represent : (1) an alteration of character in an originally agglutinable cholera vibrio, perhaps due to symbiosis with other intestinal organisms or to growth under conditions of advancing immunization ; (2) only an apparent alteration due to increasing preponderance of inagglutinable cholera vibrios, which were only present in very small numbers originally ; (3) associated vibrios which are not to be regarded as in any way causal in the disease, are possibilities to be considered.

Another observation relates the appearance of inagglutinable or less agglutinable vibrios to declining infectivity and to the subsidence of an epidemic. For a declining epidemic, Zlatogoroff (1911) gives the figures 39 out of 260 cases investigated showing lower agglutinability than at the beginning of the epidemic and 7 showing no agglutinability at all ; yet none of the vibrios differed in any respect from the true cholera vibrio except in this matter of agglutinability. Greig (1917[1]), for strictly clinical cholera stools, examined once or oftener if negative, found :

Cholera vibrios only	Cholera-like vibrios only	Both vibrios	No vibrios of any kind	Total
506	51	30	72	659

This feature of cholera vibrio inagglutinability may be only a temporary character. The several modes of treatment which may restore agglutinability are subculture, passage through guinea-pigs or combinations of subculture and passage, preservation of the vibrio in an agglutinating serum, symbiosis, repeated freezing and thawing, &c. Sometimes one method succeeds where another fails, but the transformation is itself sufficiently good evidence for the view that the weakly agglutinable or inagglutinable vibrios in clinically cholera cases may be true cholera vibrios. The injunction, therefore, to put an inagglutinable vibrio through various tests before regarding it as permanently inagglutinable, or another species of vibrio, is very necessary, and also the injunction to regard all cholera-like vibrios isolated in times of cholera prevalence as rendering the individual harbouring them suspect.

The views of Zlatogoroff on the occurrence of transformation of cholera vibrios into inagglutinable vibrios are shared by Horowitz (1911) who, however, adds certain important opinions in regard to these atypical cholera organisms. According to her, atypical cholera vibrios are nothing

but individual vibrios in a state of transition and not in any sense degenerative forms. Conditions which may lead to reversion towards saprophytism are the development of immunity in the infected individual and symbiosis with excremental organisms. These conditions are conceived of as leading to replacement of the originally preponderant, wholly parasitic strain by the incompletely parasitic strain, which, although present, was scarcely capable, through paucity of numbers, of isolation at the outset of the disease. The views of Zlatogoroff and Horowitz are again directly opposed by Wankel (1912), who, following their technique, found it impossible to transform either a true agglutinable cholera vibrio into an inagglutinable atypical vibrio, or to convert a cholera-like vibrio into a typical agglutinable cholera vibrio. The adoption of any view of the saprophytism of the cholera vibrio implies a belief in the possibility of its extracorporeal existence. A belief in this is frankly expressed by many authors.

Tomb and Maitra (1926) have affirmed that the inagglutinable vibrios found in village tanks in Bengal during epidemic prevalence of cholera owe their presence there to the use of these tanks by human beings for purposes of washing after defæcation, and that they disappear either by the closure of a tank to washing or with the subsidence of the epidemic. They further identify the inagglutinable—presumably less virulent and more saprophytic—vibrio as the causal agent in the sporadic occurrence of cholera.

So far, the only positive conclusion we have been able to reach is that the definitely agglutinating vibrio obtained from truly clinical cases of cholera in epidemic times is a true cholera vibrio. The position as regards atypical forms is still unsettled. The extracorporeal condition of existence of the cholera vibrio which demands most attention, apart from artificial culture, is that of sojourn in water. The problem is one of the highest importance for a disease for which there is so much evidence that it is largely water-borne. The large majority of cholera-like vibrios isolated from water have been inagglutinable. Has, then, experimental work upon the effect of residence in water upon agglutinability of the known cholera vibrio shed any light upon the question of the identity or non-identity of these cholera-like vibrios with the true cholera vibrio ?

Zlatogoroff found that cholera vibrios subpassaged in water and kept at temperatures of 10 to 4° C. showed steady diminution of agglutinability, which might fall from 1–10,000 to as low as 1–400. But these same vibrios could have their agglutinability restored again by cultivation in solid media. Barrenscheen (1909) confirmed these results, but Köhlisch (1910) and Haendel and Woithe (1910) failed to do so. The latter authors, with 5 passages of the cholera vibrio through water and a total interval of 71 days, were unable to find a diminution in agglutinability. Stamm (1914) took up the same problem with 13 true cholera cultures. Of these, 7 showed considerable change after 14 to 19 passages, whilst the remaining 6 cultures showed no change at all even after 21 passages. The vibrios

with agglutination diminished showed other loss of characters—gelatin was only slowly liquefied or not at all; no indole was produced and virulence was lost. On the other hand, one character was retained by which these transformed atypical vibrios could at once be differentiated from non-cholera vibrios and so to speak kept within the species; they retained specific agglutinogenic power. The general deduction which may be made is that some serological transformation seems to be effected by sojourn in water, that it is not easily effected and that it is incomplete but may be permanent as far as it has gone.

The evidence for transformation to saprophytism is still rather slender, but light is thrown on the problem by the recent studies on dissociation in organisms. By dissociation is meant the adoption by an organism of a form which differs significantly in morphological, cultural, serological and infectional characters from its original form. It is usually effected by subjecting the original type of organism to the action of adverse circumstances. The features of the new type of organism from the serological point of view are spontaneous agglutinability in normal salt solution, diminution or alteration of agglutinability by standard sera, increased phagocytability, diminished virulence and diminished protective character. The type of colony formed may also be modified.

Balteanu made search for these variants in the case of the cholera vibrios by the methods which have been found successful for the purpose. He obtained three types of colony variant—circumvallate rugose, white ring, and opaque colony forms, but restricted himself in his serological analysis of antigen to the last of these for comparison with the original ordinary smooth colony type of cholera. This opaque variant was derived from a hæmolytic strain of *V. choleræ* with the ordinary morphological and cultural characters. It formed a tough pellicle on liquid media, was devoid of motility, and the component vibrios of its colony were surrounded by mucoid material. From a series of tests with antisera produced to ordinary typical cholera and to the opaque variant, and by the use of suspensions of these strains, heated for 2 hours at 100° C. and unheated, Balteanu has deduced that the typical cholera vibrio, like the *B. proteus* used by Weil and Felix, exhibits double antigenic character. These antigens are heat-labile and heat-resistant respectively, and resemble the flagellar and somatic antigens of Smith and Reagh (1903). Special means had to be taken for the cholera vibrio, which has only a single flagellum, to bring out the loose flocculating character of the heat-labile antigen, which consisted, in the addition to the vibrios under test, of a suspension of flagella. The opaque non-motile variant, when subjected to test, gave evidence only of the existence of the heat-stable, that is, non-flagellar or somatic antigen, and was precipitated only in compact granular form. It seems, however, that this variant was not an absolutely pure form because the antiserum furnished by it could be shown still to contain some heat-labile antigen, both by absorption tests, and by a degree of flocculent clumping of flagellar suspension.

To sum up Balteanu's work : The clear translucent type of colony of *V. choleræ* consisting of motile vibrios, the normal type, appears to possess a double antigenic structure. In old cultures of this normal cholera vibrio in fluid media there is developed a variant giving opaque colonies of non-motile vibrios ; this non-motile vibrio exhibits only single antigenic capacity, that which gives, like the thermo-stable of the two antigens of the normal type, agglutination in small compact granules, with an agglutinating serum.

Balteanu, then, did not develop a variant with inagglutinable characters to cholera agglutinating serum. Shousha (1924), on the other hand, seems to have developed what was in some respects a more nearly pure rough (R) colony variant in the sense of Arkwright. It was, however, still motile and, therefore, was also not wholly pure. This variant, which like that of Balteanu, was developed from a hæmolytic strain of vibrio, showed only slight cross agglutination with the original.

Serological Methods.

These are in no way peculiar to cholera and need not be described in detail.

Agglutination. The tests which are used in practice are the rapid slide test and the test for limits of agglutinability. In the rapid slide test, the organisms from a colony or from a pure culture on slope agar are suspended in pure agglutinating serum or in a low dilution of such serum. It is designed to pick out, as rapidly as possible, by means of macroscopic agglutination, the colonies which are likely on further examination to be colonies of the cholera vibrio. The second test, which may be called the end titre test, may be microscopic or macroscopic, and may be used for the identification of the cholera vibrio or of serum derived from a cholera case. Suspensions of vibrios for this test are made by rubbing up a portion of a slope agar culture in normal salt solution, in 0·1 per cent. formolized normal salt solution, or more simply still by adding 0·1 per cent. formol to a 24-hour broth culture. The formalin hardens and fixes the bacteria as it would a tissue and there is no liability to autolysis. High titre sera should be as potent as possible and certainly not of less titre than 1 in 1,000. Rabbit sera are used in preference to those of other animals because they do not normally agglutinate cholera vibrios to any extent. Mackie (1922) and Greig both used plain saline suspensions for test while Douglas (1921) strongly recommends formolized broth cultures.

In speaking of agglutination to titre, some degree of latitude must be allowed from exact correspondence of the end points. Baerthlein (1912) allows, for example, for a 1 in 5,000 cholera serum, a variability from 1 in 2,000 to 1 in 5,000. The serum of cases of cholera which have not died too rapidly for agglutinins to appear, and the serum of convalescents may be tested in exactly the same way as in the test for identification of the vibrio.

Bacteriolysis. These methods very largely centre, for the cholera vibrio, in the Pfeiffer reaction, which is carried out with an immunized animal, or more commonly with an immune serum and a non-immunized animal.

Complement fixation. This test is carried out in exactly similar fashion to that with any bacterium.

Serum Reactions in Identification.

The various marks by which the cholera vibrio is identified—liquefaction of gelatin, fermentation of carbohydrates (glucose, maltose, saccharose, dextrin), absence of hæmolytic power, pathogenicity, even when taken collectively are not wholly sufficient to characterize this organism. It requires the positive, serological test to make the diagnosis absolute. Nor does a negative serum test immediately exclude the possibility that the test organism is a cholera vibrio. Not until a series of subcultures have been effected; not until the test organism has been tried in its capacity of agglutinogen, and not until, according to recent serological research, it has been tested with pure line antisera corresponding to S, O or R antigen, can it be conclusively decided that it is not a cholera vibrio but a cholera-like vibrio.

Serum Reactions of Infected and Convalescent Patients.

In cholera the disease is very rapid in its development and usually well-defined clinically. The immunity response, as evidenced by serum reaction, has scarcely developed before the disease is over. The following tables have been extracted from the data given by Greig for the first 12 days of the disease:

Table I.

Fatal Cases.

Number of days of disease	No agglutination	Agglutination in dilution of 1 in:					Totals
		20	40	80	100	160	
1	3						3
2	19						19
3	16	2	1				19
4	11	2	2				15
5	6	3	4				13
6	1	2					3
7		3					3
8			1				1
9	1	1					2
10						1	1
11							0
12	1						1
Totals	58	13	8	0	0	1	80

TABLE II.

Non-fatal Cases.

Number of days of disease	Agglutination in dilution of 1 in :													Totals
	Nil	20	40	80	100	160	200	250	300	400	500	800	1000	
1	2													2
2	24	2	3	1										30
3	29	9	4	2			1							45
4	23	21	11	1						1				57
5	12	15	14	3	2					1				47
6	2	13	16	7			3	1	1	3			1	47
7	3	8	12	14	1		3		1	1				43
8		6	9	2	2	1	3		1	2			1	27
9		2	4	5	1		2		3	3			1	21
10		2	2	1	1	1	1						3	11
11		1	2	4	2		2	1	2	2		1		17
12			2		1		1							4
Totals	95	79	79	40	10	2	16	2	8	13	0	1	6	351

There is very little development of agglutinin in fatal cases of cholera for the reason that the time for such development is insufficient and, where development has taken place it rarely exceeds 1 in 40. From the table of non-fatal cases we find that agglutinins may manifest themselves in a few cases as early as the second or third day of disease, but not in any large number of cases, nor in any high degree till the fifth or sixth day, after which they are increasingly evident and may obviously be used, if necessary, to confirm a clinical or bacteriological diagnosis. Titres as high as 1 in 1,000 may be reached. The recovered case serum has a further definite use in so far as it reacts with the organism, isolated from the stools, which is causally related to the affection. In this way it is possible to settle, for example, that an isolated cholera-like vibrio, reacting with the patient's serum, is the true cause of a choleraic affection and not *V. choleræ* itself.

The occurrence of a definite agglutinating reaction in carriers has been recorded by Greig. Sakai (1917) records for 84 carriers that one-third had a titre of over 1 in 200, and that the highest titre he obtained was 1 in 2,000. On the other hand, healthy carriers and contacts have been shown not to furnish an agglutinating serum (De Bonis, 1912).

Pathogenic Action of Vibrio Choleræ.

By E. D. W. Greig.

Pathology of Infection.

Small Intestine.

The condition of the small intestine first arrests attention in cholera. *Post mortem* the contents are, as a rule, clear fluid in which mucus and flakes of epithelial tissue are observed. This fluid is passed by the patient during life, and forms the characteristic ' rice water ' stool. It is vomited also in large amounts. Sometimes the fluid is reddish in colour due to the addition of blood. Scicluna (1913), at Malta, records some severe hæmorrhagic cases amongst 116 observed. In other cases the contents may be thick like pea-soup. In very acute cases there is a reddish fluid with numerous pale red mucous patches floating in it, resembling coarsely cut flesh which has been washed in water and expressed. In more chronic cases marked changes are noted in the mucous membrane of the small intestine ; the surface layer is detached, the submucosa is reddened and inflamed, especially at the border of Peyer's patches. The blood-vessels in the wall of the intestine are injected. Microscopical sections show that the *V. choleræ* has penetrated into the submucosa. Necrotic changes are noted in the mucous membrane, especially in portions near the ileo-cæcal valve. It has to be remembered that cholera may be complicated by dysentery or typhoid (Stoerk, 1916).

V. choleræ *in contents of small intestine.* In acute cases, films made from the contents show, as a rule, practically a pure culture of *V. choleræ*. In more chronic cases other bacteria may also be seen.

The great loss of fluid from the alimentary tract of cholera cases causes marked dehydration of the tissues, which explains certain symptoms, e.g. muscular cramps, concentration of blood, fall of blood-pressure, and post-mortem appearances such as dryness of tissues, &c.

Gall-Bladder and Biliary Passages.

Next in importance to the lesions of the small intestine are those of the gall-bladder and biliary passages. Kulescha (1910), Sewastianoff (1910), Greig (1913[2]), Coulter (1915), Schöbl (1916) and Crowell and Johnston (1917) directed special attention to the changes met with. Thus, Greig (1913[2]) examined 271 fatal cases and found that 80 gave a pure culture of *V. choleræ* in the bile. Further, 12 out of these showed definite naked-eye signs of cholecystitis. Microscopical examination demonstrates that the epithelial layer has been partly detached, and that the *V. choleræ* has penetrated into the submucosa. In the bile the cholera vibrios find very suitable conditions for their prolonged life ; the bile has an alkaline reaction and other organisms are usually absent—conditions which are in marked contrast to those found in the digestive tract. Schöbl (1916) from his observations, considers bile is a good medium for

the growth of *V. choleræ*. The observations on the gall-bladder explain the mechanism of the important 'carrier' state in man. As in typhoid fever, so in cholera, the organism causing the disease can continue to live in the gall-bladder for long periods without causing observable signs of ill-health. It is discharged with the bile into the intestine and so gains access to the external world. As will be seen later, the discharge is intermittent.

Liver.

Greig (1914[2]) observed cellular infiltration round the bile ducts with distension of capillaries. Also the liver cells, especially near the gall-bladder, showed degenerative changes, the cells staining badly. Kulescha (1909) reports liver abscess due to *V. choleræ*. Yamabayashi (1914) notes that the liver cells of the rabbit, after administration of *V. choleræ*, show cloudy swelling. Diatroptoff (1894) and Greig (1914[2]) found cholera vibrios present in the liver of cholera cases *post mortem*.

Kidneys.

Important changes occur in the kidneys, especially in prolonged cases; the lesions are parenchymatous, the cortex shows fatty degeneration, and the pyramids are hyperæmic. Utsumi (1922) records degeneration and necrosis of the tubules and more or less degeneration of the glomeruli. Sellards and Shaklee (1911) associate the serious condition of uræmia with acid intoxication. Greig (1913[4]) cultivated *V. choleræ* from the kidney and urine.

Lungs.

An important and serious complication of cholera is pneumonia. In these cases *post mortem* small areas of pneumonic consolidation with a diffuse congested zone round them are observed. Greig (1913[2], 1914[2]) examined these areas microscopically and found the alveoli filled with cellular exudation in which *V. choleræ* could be seen, and it was cultivated from the nodule. Stoerk (1916) reports pneumonia as the commonest complication in cholera, and Utsumi (1922) also has recorded a case. Diatroptoff (1894) isolated *V. choleræ* from the lungs in all of five cases examined.

Lymphatic Glands.

Enlargement of these glands, especially near the intestine, has been noted. Greig (1914[2]) cultivated *V. choleræ* from them. This is important as appearing to indicate that the organism is distributed by the lymph rather than the blood-stream.

Nervous System.

Bechterew (1910) has observed masses of *V. choleræ* in the brain. Scicluna (1913) records a case of ordinary acute meningitis without intestinal lesion due to the *V. choleræ*. Michailow (1912) records

degenerative changes of the nervous system in cholera. Utsumi (1922) found that the diaphragmatic nerves especially, showed segmentation of the axis cylinder.

Heart.

Manwaring, Boyd, and O'Kami (1923) have shown in the excised mammalian heart, that the cholera toxin is an endotheliotoxin and acts on the endothelium, and has no action on the muscle. Utsumi (1922) noted waxy degeneration in the heart.

Skin.

A rash occurs in cholera and there are frequent references to it in the literature—Artz (1914), Scicluna (1913), Ichikawa (1916). The latter describes several types of rash, not only on the skin, but also on the mucous membranes, which appear between the seventh and twelfth day of the disease. A possible explanation of the rashes may be that they are anaphylactic phenomena accompanying the development of immunity against the cholera toxin.

Bone-Marrow.

Kulescha (1910) has observed changes. The yellow marrow becomes red either in its whole thickness or in patches. There are two stages: (1) Erythroblastic reaction to the deficiency of oxygen in the blood, and (2) granulo-cellular hyperplasia, being the reaction to the infection itself.

Distribution of *V. choleræ* in the Body.

Although chiefly found in the small intestine and biliary passages, *V. choleræ* has a more extensive distribution: generalized infection has been noted by various observers—Diatroptoff (1894), Bordoni-Uffreduzzi and Abba (1894), Sewastianoff (1910), Greig (1914[2]). The latter systematically examined the organs in a series of cases for *V. choleræ*. Some details of the observations are given below. One of the cases, No. 185, lived for five days after the onset of cholera and died of uræmia.

Table III.

The result of the bacteriological examination of the stools and urine of case No. 185:

Name	Age	Sex	Caste	Number of days to death	Date of examination	Presence or absence of *V. choleræ*	
						Stool	Urine
Haridasi ..	30	F.	Hindu	6 days	3rd day	+	—
					4th day	+	+
					5th day	+	—

TABLE IV.

The organs bacteriologically examined of case No. 185, and presence or absence of *V. choleræ :*

Organs examined bacteriologically	Presence or absence of *V. choleræ*
1. Right lung (pneumonic area, lower lobe)	+
2. Left lung (pneumonic area, lower lobe)	+
3. Trachea	−
4. Heart-muscle : portion wall of left ventricle	−
5. Gall-bladder :	
(*a*) portion of wall	+
(*b*) bile	+
6. Liver	+
7. Spleen	+
8. Kidney, right	+
9. Kidney, left	+

PORTAL OF ENTRY OF *V. CHOLERÆ.*

The natural channel is by the mouth, and the vibrio is ingested with food or water. As is well known, *V. choleræ* is very sensitive to acids and a great many are killed in the stomach, the acid gastric juice acting as a natural barrier against invasion. Drinks, especially if cold, pass rapidly through the stomach into the intestine. Schutz-Schultzenstein (1901) noted that 600 c.cm. of water given on an empty stomach and removed 12 to 15 minutes after introduction gave an acidity 0·03 per cent. HCl. This kills *V. choleræ* in 15 minutes. Water containing albumin or peptone requires much more acid to kill the vibrio. Straus and Wurtz (1889) observed that *V. choleræ* was killed in 2 hours by the gastric juice of man, dogs and sheep. Hamburger (1890) found that gastric juice containing free acid was sterile and that *V. choleræ* was killed by it with certainty. Sometimes the vibrio may be in the interior of masses of food, and so pass the stomach unacted upon by the gastric juice. Further, in some persons the free acid is small in amount, or even absent.

Having passed through the stomach, *V. choleræ* finds in the alkaline intestinal juices a much more favourable medium and multiplies rapidly. Sanarelli (1923) goes so far as to say that when taken *per os* it is killed by the acidity of the stomach and does not reach the intestine. In his opinion the vibrios deposited on the buccal mucous membrane or insufflated into the nasopharynx pass into the blood-stream and are carried by this route to the intestine. Sanarelli (1922) has shown that young dogs up to 3 or 4 days are very susceptible to infection. When given *per os V. choleræ* leaves the stomach and passes into the blood-stream, where it multiplies and ultimately reaches the intestine. The

serum of dogs only attains vibriocidal capacity 3 to 4 days after birth. Probably this applies to all young animals. Metchnikoff (1894) considers that in the immunity of man and animals to cholera, intestinal organisms play an important role. Kempner (1895) discusses the antagonism between *V. choleræ* and *B. coli*. Pane (1912) regards *B. pyocyaneus* as the only fæcal organism capable of inhibiting *V. choleræ*. Romano (1912) points out that the intact intestinal epithelium affords considerable protection from invasion by the latter organism.

Undoubtedly errors of diet play an important part in assisting the cholera vibrio by producing paralysis of peristalsis, alteration of distribution of blood, and lastly, by direct damage to the intestinal epithelium of the mucous membrane and lowering its resistance. Hence the importance of avoiding, during prevalence of cholera, indigestible food, drinking excessive quantities of cold water, &c.

Discharge of *V. Choleræ* from the Body.

Stool.

This is the most important exit; a less frequent one is the urine. Systematic observations on the discharge of *V. choleræ* in the stools of convalescents and contacts were made by Greig (1913[1]). The observations were frequent and carried on over a prolonged period. In this way it was possible to follow exactly the discharge of the vibrio. Some of the results of the investigation are set forth in the following table:

Table V.

Name: B.M., Hindu, Male.
Age: 27.
Date of attack: 31st July, 1912.

Date of examination. Stools	Widal reaction. Blood. *V. choleræ*	Presence or absence of *V. choleræ* in stools	Character stool	Body weight, lb.
1912.				
July 31 ..		—		110
Aug. 1 ..		+		
3 ..		+		
4 ..		—		
5 ..		—		
6 ..		—		
11 ..		—		95
12 ..		—		
18 ..		—		
19 ..		—		
20 ..		—		
21 ..		—		
22 ..		—		

TABLE V—*cont.*

Date of examination. Stools	Widal reaction. Blood. *V. choleræ*	Presence or absence of *V. choleræ* in stools	Character stools	Body weight, lb.
1912—*cont.*				
Aug. 23 ..		—		
24 ..		++	Soft (with mucus)	90
25 ..		++		
26 ..		—		
27 ..		—		
28 ..		—		
29 ..		—		93
30 ..		—		
31 ..		—		93
Sept. 1 ..		—		
2 ..		Vibrios (Non-agglut.)		
3 ..		—		
4 ..		—		
6 ..	± + + — — (10 30 60 90 120)	-		
7 ..		—		
8 ..		—		98
9 ..		-		
10 ..		—		
11 ..	+ + ± — — (10 30 60 90 120)	—		
12 ..		++		
13 ..		++		
14 ..				
15 ..	± + ± + ± (10 30 60 90 120)	—		
16 ..		-		
17 ..		—		
18 ..	± ± + ± ± - (10 30 60 90 120 200)	—		
19 ..		—		
20 ..		—		

Remarks. This is an important case. *V. choleræ* was recovered from the stools six weeks after the attack. The discharge of the vibrios was markedly intermittent. It is noteworthy that cholera-like vibrios occurred in the stools of this man on one occasion, but they did not react with high titre cholera agglutinating serum. His blood also contained cholera agglutinins.

Urine.

Greig (1913[4]) examined the urine of 55 cases and in 8 cultivated *V. choleræ* from it. Sewastianoff (1910) has also cultivated the organism

from the urine. The exit of *V. choleræ* from the body by the urine is of occasional occurrence only, but, nevertheless, it cannot be entirely ignored.

Evidence of Ætiological Relationship of *V. choleræ* to Cholera.

Production of Cholera in Man.

Accidental. Classical examples are the following : In 1884, at Koch's course in Berlin, a medical man contracted cholera. No other cases of cholera were reported in Germany at the time. Pfeiffer and Pfuhl contracted cholera in pretty severe form and *V. choleræ* was found in the dejecta. No other cases of cholera were occurring in Berlin.

Intentional. Dunbar (1896) used avirulent cultures, and the results were negative. Pettenkofer and Emmerich (1892) swallowed cultures. Pettenkofer only had light diarrhœa without other toxic symptoms, but Emmerich developed an attack of cholera. *V. choleræ* was found in their dejecta. The incubation period was 12 to 48 hours.

Cholera Epidemics on a Large Scale.

These have furnished in nature much more convincing support of the ætiological relationship of *V. choleræ*. This question will be dealt with in detail under ' Epidemiology ' on page 390. Cunningham's (1891) view that *V. choleræ* may not be the cause, but that the disease may be the reason for its presence, has long since been disposed of.

Animal Experiments.

These have supplied also valuable evidence of the ætiological relationship, and will be referred to later in detail.

The constant finding of true *V. choleræ* in all cases of acute disease and ' carrier ' state and the evidence afforded by specific serological reactions have placed the ætiology of cholera on a very firm foundation.

Toxicity.

Endotoxin of Cholera.

Cantani (1886) gave experimental proof of an endotoxin in cholera. Pfeiffer and Wassermann (1893) showed that a filtrate of a 1 to 5 days' broth culture of *V. choleræ* contained little poison. Fresh agar cultures, even when quite young and killed by heating for 1 hour at 56° C., were lethal to guinea-pigs of 200 gm. in such small quantities as 10 mgm. when injected intraperitoneally. Also, when the *V. choleræ* was killed by agencies such as strong chemicals, boiling or prolonged heating to 80 to 90° C., the toxic action became less specific, because the primary toxin (nucleoprotein) had been converted into a secondary toxin (nuclein). Their researches emphasized the fact that the toxin is closely bound up with the protoplasm of the cell and not easily liberated. Kraus (1920) believes that the cholera endotoxin produces an anti-endotoxin. Landsteiner and Levine (1926) obtained from the cholera vibrio an alcohol-soluble specific substance. It was present in hot 75 per cent. alcoholic extracts of the bacilli previously washed with ether and hot absolute alcohol. A

sediment separated on cooling which reacted with anticholera serum up to 1–500,000 dilution of the antigen, gave a protein reaction, and acted as an antigen on injection. Further purification gave a specific substance containing carbohydrates, which failed to give a biuret reaction and had slight antigenic capacity, but reacted with immune serum in a dilution of 1–500,000. The original antigenic activity could be explained if the alcohol extract contained a complex antigen of protein and a specifically reacting but non-antigenic substance.

Exotoxin.

Although the German school consider that cholera toxin is essentially an endotoxin, other investigators, particularly French, are of opinion that *V. choleræ* also excretes a toxin *in vitro* and *in vivo*. Metchnikoff, Roux, Taurelli-Salimbeni (1896) placed cultures in peptone water in collodion sacs and introduced them into the peritoneal cavity of guinea-pigs. They were left for a long time and the animals died with toxic manifestations. Kraus (1909) states that the cholera vibrio produces a soluble toxin, and an antitoxic serum can be prepared which acts on the strain antitoxically, but bacteriolytically both on cholera and cholera-like vibrios, e.g. the El Tor vibrio. Bail (1917), using a special strain (Kadikjo) of *V. choleræ*, obtained watery extracts which produced antitoxic serum, which worked to laws of multiple proportion. This toxin does not represent the true toxin of the bodies. Takano (1918) compared various solutions obtained by washing killed cholera or live cholera vibrios and the residual bacterial body. He did not find any difference in the various immunological reactions and the symptoms produced in guinea-pigs with these solutions, and he came to the conclusion that it is difficult to distinguish in cholera between the exotoxin and the endotoxin. When the vibrio dies, the main constituents of the bodies permeate into the medium, and in all liquid media the vibrios are continually dying. Consequently, the finding of toxin in the medium is not sufficient evidence of the existence of an exotoxin. Acton and Chopra (1924) consider that the cholera vibrio forms toxins in broth cultures from the amino-acids, chiefly in the arginine fraction ; but Kolle and Prigge (1927[1]) say that this view has not been confirmed by others, and that the antigenic properties of the material are not even stated, so that the connection with true cholera toxin is questionable.

Only a brief reference is necessary to the theory of Emmerich (1911), who regards cholera as a nitrite poisoning. Kolle and Prigge (1927[2]), regarding this theory, state that other pathogenic organisms (*B. paratyphosus* B, *B. dysenteriæ* (Flexner), &c.) which produce clinical manifestations totally different from cholera, are as good nitrite producers as the cholera vibrio. This appears to dispose of the nitrite poisoning theory.

It will be seen from the above that much more work is required on the toxin of cholera.

Mode of action of cholera toxin. Sanarelli (1924), as a result of prolonged experimental studies, concludes that cholera toxin has a special attraction for epithelium, and causes a shedding of the epithelium of the intestine and gall-bladder, and also an anaphylactic action. Cholera toxin produces in grown rabbits appearances which, clinically, microscopically, macroscopically, bacteriologically and hæmatologically are those of anaphylactic shock, and also the appearances of cholera.

Hæmolysin or Hæmotoxin.

This subject has been discussed fully on p. 364, and it is not necessary to deal with it here again. It may be mentioned that Greig (1915[3]) made a large number of observations on the hæmolytic action of true cholera and cholera-like vibrios. Three hundred and thirty-three strains of typical freshly isolated cholera vibrios from acute cases of cholera in Bengal were tested, and all were non-hæmolytic. So that among the Indian strains tested by the technique given, none resembled the El Tor or Nasik strains. The absence of the El Tor vibrios from acute cases, and its association with cases in which the clinical signs have been much less severe than those of ordinary cholera, suggest that this organism is of a lower order of pathogenic activity than the true *V. choleræ*. One hundred cholera-like vibrios, on the other hand, isolated from various sources were tested and the great majority were hæmolytic, some strongly so. This was in marked contrast with the results obtained with the cholera vibrio.

Experimental Observations on Animals.

Cholera does not occur naturally among animals, but much experimental work has been carried out on them. Nikati and Rietsch (1884) by injection of *V. choleræ* direct into the duodenum of rabbits after ligature of the common bile duct produced a disease of the epithelium of the intestine very like cholera. Koch (1885) noted the destructive action of the gastric juice, especially marked in guinea-pig, on the cholera vibrio. He devised a method of infecting animals. He neutralized the gastric juice of the guinea-pig with 5 c.cm. of a 5 per cent. solution of sodium bicarbonate introduced by tube into the stomach. Afterwards the guinea-pig received 5 to 10 c.cm. of water to which a little *V. choleræ* culture was added, and at the same time 1 c.cm. tinct. opii per 200 gm. body weight was injected intraperitoneally. Next day the animal was sick. Later it collapsed ; the body was cold ; there was weakness of the extremities ; and death took place in 24 to 30 hours. *Post mortem*, the small intestine was found to be very red, and contained a large quantity of rosy fluid with epithelial-cell tissue. Films made from the contents showed a pure culture of *V. choleræ*. Pfeiffer (1894) introduced the intraperitoneal method of infection of guinea-pigs. One-tenth to one-twentieth of a loop of agar culture constantly produced a fatal peritonitis with active development of *V. choleræ* in guinea-pigs of 200 gm. body

weight. When the minimum lethal dose was determined, the peritoneum and internal organs remained absolutely sterile. The intestines rarely contain *V. choleræ*. Sanarelli (1916) administered *V. choleræ per os* to young rabbits, and it reached the intestine via the blood-stream. Sanarelli (1924) found young rabbits exceedingly susceptible to intravenous injection of *V. choleræ*, but that the adult rabbit is more resistant. The former react to subminimal doses by a gastro-enteritis, in which the blood and organs are sterile, but *V. choleræ* is found in the whole extent of the gastro-intestinal tract (gastroenterotropism). He believed also that the vibrio injections in animals led to a sudden mobilization with increase in virulence of intestinal bacteria, especially *B. coli :* further, that *B. coli* entered the blood and produced a marked septicæmia. He endeavoured to explain this phenomenon. He injected sublethal doses of vibrio, and, 24 hours later, harmless quantities (1 to 2 c.cm.) of colitoxin. In this way he often produced severe collapse, which he regarded as an anaphylactic crisis. Sdrodowski and Brenn (1925), referring to Sanarelli's work, state that they have produced the same syndrome in rabbits without *V. choleræ*, namely, by small intravenous injections of *B. coli*, and, 24 hours later, intravenous injection of a non-toxic dose of *B. coli* filtrate.

Injected into the breast-muscle, true *V. choleræ* is very slightly virulent for pigeons, but cholera-like vibrios are much more active than the true cholera vibrios and produce fatal septicæmic infections. The injection of the pigeon intramuscularly has been used by Pfeiffer and Nocht (1889) and Gotschlich and Weigang (1895) to differentiate between the two.

Greig (1914[2], 1915[2&3], 1917[2]) carried out an extensive series of experiments with animals. Following intravenous injection of *V. choleræ* in adult rabbits, some developed symptoms of cholera, and in some a pure culture of *V. choleræ* was obtained from the bile, heart-blood, and intestine ; others were negative. The symptoms in the infected rabbits were similar to those of a severe case of cholera in man. Very loose stools were passed and the animal rapidly passed into a state of collapse and appeared very sick. The strains of cholera vibrio used were obtained directly from man, having been cultivated from the bile or stool. The quantities injected varied from 0·25 to 2 agar slopes. *V. choleræ* was cultivated *post mortem* in some cases from the bile, the heart-blood and the intestinal contents, in others no growth was obtained from these sources.

Pathological Changes.

The pathological changes found in one rabbit *post mortem* were as follows : The continuity of epithelial layer of the gall-bladder wall was destroyed at one point, and there was a considerable increase of round cells, poly- and mononuclear types, in submucosa. Round the intrahepatic bile ducts there was much cellular exudation, and the blood capillaries were considerably distended. In the duodenum the epithelial layer was partly gone and there was an increase of the cells in the villi ; the blood-vessels were distended. *V. choleræ* was seen between and inside cells.

Strains of cholera-like vibrios were used also. The virulence of these was much greater than that of the true *V. choleræ*. The various organs were invaded.

'Carrier' Condition produced Experimentally.

Greig (1916) immunized a large number of rabbits with vibrios, true cholera and cholera-like, from various sources, by intravenous injection of the living organism. Certain of the rabbits died at varying intervals after the last dose of living *V. choleræ*, and, *post mortem*, pure cultures of *V. choleræ* were obtained from the bile and in some cases definite signs of cholecystitis were evident. Thus a rabbit, No. 114, was immunized with vibrio No. 412 (a cholera-like vibrio isolated from the stool of a 'carrier'). The rabbit received its last intravenous injection of living vibrios on 17th September, 1914; it died on 16th December, 1915, and vibrio No. 412 was cultivated from its bile. It was thus shown experimentally that the rabbit had harboured the vibrio for a period of 15 months without showing obvious signs of ill-health. Partially healed ulcers were noted in the mucous membrane of the gall-bladder. Rabbit No. 121, injected with a standard *V. choleræ* (Berne), harboured the vibrio in its bile and was a 'carrier'. In several of the animals, gall-stone formation was noted.

Feeding Experiments.

Greig (1917[1]), using the technique of Pottevin and Violle (1913) (the administration by stomach tube of suspensions of vibrios after neutralization of the gastric juice), produced a fatal disease in adult rabbits with true cholera and cholera-like vibrios. The lesions were very similar in both cases. It was noted that some strains of vibrio produced only slight or no signs, and the animal recovered; other strains, on the contrary, produced rapidly fatal results. This variation in virulence probably helps to explain the known variation in severity of epidemics and individual cases of cholera. Microscopical study of serial sections of portions of the intestines of rabbits fed on vibrios showed that the epithelial layer had been destroyed, thus giving access to the submucosa. The vibrios even penetrated into the blood-stream, bile, &c.

Epidemiology and Spread of Infection of Cholera.

By E. D. W. Greig.

Older Views of Epidemiology.

Pettenkofer's Theories.

Pettenkofer (1886, 1892, 1894) found that cholera was never uniformly distributed with the same intensity. He concluded that epidemics depended on: (1) Seasonal conditions.—In Germany, cholera epidemics spread mostly in late summer or autumn; they are least in February, March, April and May. In India, e.g. Bombay, the minimum period occurs from June to September, the maximum from February to May. (2) Local conditions.—After the discovery of the cholera vibrio

by R. Koch, he modified his theory and attributed the unique causal role to it—*V. choleræ* = X.—For occurrence of cholera, required X + Y (unrecognized factor). Y was bound up with locality, viz. (1) special humidity, and (2) degree of contamination of soil. The substance resulting from the union of 'X' and 'Y', Pettenkofer called 'Z'.

Cunningham (1897), a student of Pettenkofer, found during the cholera season in India that *V. choleræ* was present in the stools of healthy persons and considered that the cholera state was produced by various vibrio species (commensalism of organisms in disease). This work was done, of course, in the preserological period of bacteriology, and subsequent investigations with modern technique explained the apparently confused conception of Cunningham. Much of the older work is now only of historical interest.

The views of Pettenkofer on the epidemiology of cholera have been collected recently by Hume (1927) in convenient form, and for further details this publication may be consulted.

Koch's Theory.

R. Koch (1884, 1885, 1893) associated the cholera vibrio, which he discovered, with cholera dejecta, and, less frequently, with the vomit. To infect man it has to be taken *per os*, and then it passes into intestine. Transmission is either (*a*) direct, by hands of sick or healthy—Koch and Gaffky (1887); or (*b*) indirectly, by foodstuffs, flies or water: the latter plays an important role. According to Koch, there is no connection with soil.

Contact infection. This occurs from person to person, especially of the poorer classes. The theory of contact infection is supported by the preventive effect of personal prophylaxis. The curve of a contact epidemic is flat; the cases occur in single groups, and cholera foci form as in 'cholera houses'. These characters distinguish it from the water epidemics.

Water epidemics. In a considerable number of cases the recovery of *V. choleræ* from water has been reported. Koch (1884) first recovered it from the water of a tank in India which was used by a population suffering from cholera. Its recovery from the water of streams, wells, ponds has been reported by Koch (1893), Lubarsch (1892), Biernacki (1895), Ströszner (1911), and others. It has to be remembered that these early observations were made without the advantages of serological methods, and, therefore, cannot carry the same weight as those made later.

The curve in water epidemics is of the 'explosion' type, with steep ascending and descending limbs. The causes of the steep descending limb are: (1) disappearance of the cholera vibrio from the water; and (2) warnings issued to the population not to drink the water. The water epidemic cases follow the distribution of water supply and the connection between cases fails. Although a pure contact and water epidemic give quite different curves, yet the two may become interlocked, a water shading into a contact and a contact suddenly becoming a water epidemic owing to water supply having become infected.

Two Classical Water Epidemics.

(1) *Nietleben* (1892–3). This epidemic occurred in the asylum at Nietleben, and was investigated on the spot by Koch. *V. choleræ* was cultivated from the raw water of a small water plant, which was open and not functioning on account of the extreme cold. Thence it reached the water mains. Cases of cholera developed after drinking the water, and ceased after the supply was abandoned, and the patients given pure water.

(2) *Hamburg.* In 1892, 9,000 deaths occurred in a few months. In that year cholera was present on the eastern and western frontiers of Germany, and in August the first cases occurred, but only among workers in the dock at Hamburg. For a few weeks single cases were reported, then on 20th August, an ' explosive ' outbreak all over Hamburg occurred, and steadily increased to the end of the month, when there were 1,000 cases in the 24 hours. At that time, Hamburg had unfiltered water taken from the Elbe by canal, and it was thought that *V. choleræ* was carried into the canal intake by tidal movements : it had possibly been imported by Russian immigrants. Altona and Hamburg had different water supplies, and many examples of peculiar distribution arose. Thus, in a street on one side, which was supplied by Hamburg water, cases of cholera occurred, whilst on the other side supplied by Altona water, there was none. Apart from the water supply, the two sides of the street had exactly similar conditions as regards soil, sewage, air, sun, &c.

In regard to ' water ' epidemics, the remarks of Houston (1913) (vide p. 397) are very instructive.

Recent Studies in Epidemiology.

General.

Webster (1923) in his precise studies, determined the interaction of three quite constant factors, namely, the level of virulence of the bacilli, the quality of resistance of the host, and the dosage or quantity of bacilli ingested. Flexner (1926) considers that the preponderant role of dosage and fresh infectible host material is clearly indicated in prolonging epidemics and explaining the periodic and wave-like movements. That spontaneous epidemics in mice are prolonged in an identical manner by new births, and by the spatial and quantitative distribution of bacilli may be assumed as quite certain. Application of these findings to the study of epidemics of human diseases in which more precise observation will take the place of actual experiment is the possibility clearly indicated.

Special.

Cholera in Europe or elsewhere on the earth never originates or can originate autochthonously. This established fact forms the foundation of the whole cholera epidemiology. Another and not less important observation is that after an attack of cholera (severe or slight) cholera bacilli may occur for weeks or months in the normal dejecta.

The endemic centre of cholera has been regarded as being in India, and in a special part of it, namely, the population of the Ganges Delta : as

our knowledge of the epidemiology of cholera increases it will probably be necessary to revise our present conceptions of the endemic centre. Thus, recently, Rogers (1926) in an intensive study of the epidemiology of cholera in India, points out that the present endemic centres, as shown by the disease never having been absent for a single year in three decades, include Assam, Lower Bengal, the Eastern sub-Himalayan division of the United Provinces of N. India, S.E. Madras, together with the low-lying west coast of Bombay. All these places have the constant absolute humidity over 0·400. The epidemic centres, from which cholera has been absent for a year or more during three recent decades, include the South and West of the United Provinces, all the Punjab, Sind, Gujerat and Deccan division of Bombay and the whole of the Central Provinces. There were a larger number of epidemics in the United Provinces than Lower Bengal, so they could not all have originated from the endemic areas of Assam and Bengal indicated by Bryden in 1869.

A number of the epidemics of the United Provinces spread over the Punjab. Rogers considers that the epidemics of the Punjab are of great international importance, as it is by the overland route to Afghanistan that cholera spreads to Europe, as in the great epidemics of 1826–37, 1863–73, 1883–96.

Cholera pandemics. Table VI shows the years of occurrence, duration, and areas of spread of the various cholera pandemics (Hirsch and Haeser, cited from Kolle and Prigge, 1927[1]).

TABLE VI.

Hirsch			Haeser			
No.	Number of years	Duration	No.	Number of years	Duration	Area of Spread
1	1817–1823	6	1a	1816–1823	7	Asia, Africa.
2	1826–1837	11	b	1826–1837		Asia, Africa, Europe, America, Australia.
3	1846–1862	17	2	1840–1850	10	Asia, Africa.
			3	1852–1860		Europe, America.
4	1883–1875	12	4	1863–1873		Asia, Africa, Europe, America.
			Later Pandemics.			
5	1883–1896	13				Asia, Africa, Europe.
6	1902–1923	21				Asia, Africa (Egypt), Europe.

The great pandemic wave of 1826–37 introduced true Asiatic cholera into Europe for the first time.

The spread of cholera is effected by man himself. Its rate depends on the speed of transport : it was comparatively slow when the journey was made on foot along the roads, and more rapid with the introduction of the railways ; a still further increase in the rate may occur with the employment of fast air-service. When the speed is slow, as in road traffic, timely warnings are given by intimation of attacks or deaths at various points. When the railway or steamship is used, the first intimation may be an outbreak of cholera in the town or village to which infected persons have been rapidly transported. The opening of new trade routes has had a very marked effect on the rate of spread of cholera. Thus the Suez Canal greatly shortened the journey between India and the Mediterranean, so that a person convalescent from cholera leaving India is much more likely now to start foci in Europe than previously. Great care has been taken to prevent this.

Pilgrim traffic is a particularly important factor in the spread of cholera in India and elsewhere. Such pilgrimages occasion great movements of people in the East, many of whom harbour *V. choleræ*. Some of the important pilgrim centres are :

1. *Mecca*. This pilgrimage has particular importance owing to the proximity of the centre to Europe and the Mediterranean.

2. *Hurdwar*. Every twelfth year large gatherings take place in late March and April, when one or two million people may collect in a few days. Every gathering since 1867 has been followed by epidemics of cholera in the Punjab, which, as has been mentioned, is particularly dangerous from the international point of view. The epidemic spreads from the Punjab through Afghanistan to South Russia by trade routes. From Russia it may extend all over Europe and to America, as in pandemic 1826–37. Hence the great importance of this pilgrimage.

3. *Puri*. For India this is a very important centre, as pilgrims come to worship Jagganath from all over India.

4. *Allahabad*. Here, at the junction of the Ganges and Jumna, every 12 years extra large numbers attend at about the beginning of February. Every one of these gatherings since 1882 has been accompanied by a great rise in cholera.

5. *Other important centres* are at Nasik, Palitana and S. India, &c., &c.

Borel (1911) notes that epidemics of cholera are related to Mohammedan holy days which change throughout the Christian year. Schumburg (1894) records that the epidemic of 1892 started from pilgrims arriving by sea at Djeddeh from India. It spread over Afghanistan, Persia, and Europe. Sergent and Nègre (1914) found cholera vibrios in the stools of Mohammedan pilgrims in N. Africa : there were 3 healthy carriers out of 67 examined.

Human Reservoir in Cholera.

From all the data the importance of the human factor in the spread of cholera emerges. Consequently it is necessary to study the problem

in some detail. Greig (1913[1]) carried out an extensive series of observations. The problem was attacked at one of the great pilgrim centres in India, viz. Puri. Here the famous temple of Jagganath, with the equally famous car-pulling festival, attracts pilgrims from every part of India. Cholera, as a rule, breaks out during the festival, and this afforded abundant material for studying its epidemiology and the spread of infection. The population of Puri is roughly 50,000. It was estimated that at least 300,000 pilgrims were present on the day of the car-pulling in July, 1912. There was thus an immense concentration of fresh infectible material.

In order to determine the number of pilgrims who harboured the cholera vibrio after recovery and were about to return to their homes in various widely separated parts of India, a number of cases about to be discharged from hospital in Puri were examined bacteriologically. Out of 30 cases 11 were still excreting the cholera vibrio in their stools at the date of discharge, and, therefore, were highly infective.

A map was constructed showing the residence in India of the convalescents discharged in July–August, 1912. It shows the very wide distribution of the points to which they go after discharge from hospital, and, consequently, that the dissemination of the cholera virus throughout the length and breadth of India is taking place by the carriers from the pilgrim centre. As they reach their homes very rapidly by rail, these pilgrims are now much more dangerous than in the days of road transport ; in the latter period many of them died before they had an opportunity of infecting fresh collections of the population.

In addition to the convalescents, a number of healthy men were examined bacteriologically. They had been in close contact with cholera cases as sick attendants. Out of 27 persons showing no obvious signs of ill-health, 6 were excreting the *V. choleræ* in their stools.

For a prolonged period the stools of 11 persons convalescent from cholera were examined daily. Although in the majority of cases the excretion of the cholera vibrio appears to stop very shortly after the acute attack, yet in 3 out of the 11 it was found at intervals in the stools for longer periods. This has been discussed in detail on p. 384.

That a pilgrim harbouring the cholera vibrio can start a cholera epidemic was shown definitely by Greig (1913[2]). He made a complete study of an epidemic which occurred in a jail at Puri, due to a carrier recently discharged from hospital there. Similar epidemics are started all over India by pilgrims returning to their homes.

The carrier problem in cholera has been investigated by many workers. Babes (1914) found that usually 10 to 20 per cent. and sometimes up to 100 per cent. of cholera cases became carriers. Twenty-five per cent. excrete for 2 to 4 days only. The period of excretion is not shortened by inoculation. Franca (1911) observed vibrios in the stool 5 weeks after convalescence. In healthy carriers the excretion was from 6 to 8 days, and the agglutinins persisted in the blood at 1 : 50 for 4 months after recovery. Gotschlich (1905) in pilgrims from El Tor found cholera vibrios

in cases of intestinal derangement, dysentery, colitis, &c. ; this was the source of the famous El Tor vibrio. McLaughlin (1909) observed no carrier excreting the vibrio for longer than 20 days. Healthy carriers in epidemic period amounted to 6 to 7 per cent., and were not so dangerous as in typhoid. Zlatogoroff (1911) found the cholera vibrio in the stool one year after convalescence. Munson (1915) in a very extensive and important investigation in the Philippine Islands reports that in 30,000 examinations of contacts 526 healthy carriers were found. Water was insignificant as a cause : ' carrier ' cases were the important factor. Several chronic ' carriers ' contracted cholera. The excretion was intermittent, in one case for a year. Such cases account for the continuance of cholera. Sacquépée (1910) considers there is no known case of a chronic cholera carrier. Tanda (1911) examined 50 recovered cases. He finds from date of first symptoms that

in 5 cases	*V. choleræ*	was excreted		10 to 20	days.
6 ,,	,,	,,	,,	20 to 30	,,
1 case	,,	,,	,,	31	,,
1 ,,	,,	,,	,,	34	,,
1 ,,	,,	,,	,,	47	,,
1 ,,	,,	,,	,,	56	,,

In Japan, Takano, Ohtsubo and Inouye (1926) note that in an epidemic in 1916, Yagasaki observed the number of days of excretion of the cholera vibrio in 200 cholera cases. In many cases they were excreted up to the end of the third week, but in some, for 40 days. They state that Izawa found vibrios in the stool (healthy) $4\frac{1}{2}$ months after recovery in exceptional cases. They give also a record of the observations made at the Moji Marine Quarantine Station. They are :

Fæcal examinations	*Cholera cases*	*Carriers*
120,637	8	11
59,687	4	5
8,091	1	2
11,575	1	1

Weisskopf and Herschmann (1915) consider that carriers are very common in cholera times and of great importance, although excretion of the causative organism does not last so long as in typhoid. In 247 cases, 24 were still excreting vibrios after 20 days. They regard 5 days' quarantine as much too short.

Water is not important in the spread of cholera. In this connection see the remarks by Houston (1913) on p. 397, which very clearly sum up the position of the carrier and his relation to water epidemics. The writer agrees entirely with Houston's views on the subject.

(For observations on the production of the carrier state in animals, see p. 390).

Viability of *V. choleræ* outside the Body.

Fæces.

Greig (1914[1]) made use of 'uncultured' strains of *V. choleræ* to test its longevity in fæces. There is a tendency for 'cultivated' strains to maintain their vitality longer than 'uncultivated', as Houston (1913) showed for *B. typhosus* in Thames water under storage conditions. Greig (1914[1]) carried on his observation throughout a year at Calcutta, examining altogether 94 stools. He used the 'rice water' stools kept in a sterile flask at room temperature and protected from sunlight and evaporation. Samples were tested daily for the presence or absence of *V. choleræ*, and a negative finding was confirmed by inseminating the whole stool in peptone water. In some cases the actual number of vibrios in the 'rice water' stool was counted daily. Also, fixed quantities of the stool—1 c.cm., 5 c.cm., &c.—were placed in peptone water and incubated; the presence or absence of vibrios determined by subculturing on Dieudonné medium, and *V. choleræ* was stated to be present or absent in 1 c.cm., 5 c.cm., &c., of 'rice water' stool.

The results show that the life of the *V. choleræ* outside the human host under natural conditions in India is short. There is, however, considerable variation in regard to the vitality of individual strains. Temperature has a powerful effect on the vitality of the vibrio. Thus, as the hot season in Calcutta (endemic centre) from March to June advances, the life of the organism becomes shorter, falling from over 6 days to a minimum of rather over a day in June. On the other hand, from December to February, the cold season, vitality is greater, and the maximum duration of life (7 to 8 days) occurred in February. Again in August, when the monsoon has developed fully and the temperature has fallen somewhat, the life is longer than in the hot season (6 days). The critical months in Calcutta as regards temperature are December, January and February. The critical cholera months directly follow the low temperature months. Accurate experimental evidence affords no support to the view that the endemic prevalence of cholera depends on the vitality of the vibrio outside the body.

According to Rogers, the absolute humidity of Calcutta, which is situated in an endemic area, is always above 0·400.

Houston (1913) states that, 'So far as infectious disease is concerned the real enemy of mankind is man and the dose of the poison is such an active factor in causing infection that we ought to guard ourselves against the individual as potential concentrator of infection'. 'If the immediate cause of what are recognized as "water epidemics" in the past could be precisely ascertained, I believe in most cases it would be found that accidental infection of the supply by what is known as a "porter" or "carrier" of disease had occurred'.

The human reservoir is in a position to supply an adequate dose of poison to various distributing channels—water, milk, flies, &c., and so,

initiate epidemics of cholera. It will be seen that the problem of the prevention of cholera is, shortly stated, the protection of mankind from man.

Fresh Water.

Carapelle (1912) isolated two vibrios from drinking-water which weakly agglutinated, but did so completely after animal passage. He considers *V. choleræ* has characters of a saprophyte in water. Craster (1913), during epidemics, found non-agglutinable vibrios which became agglutinable. Dunbar (1896) believed that although the cholera vibrio undergoes great changes in water so as to be almost unrecognizable, yet he confirmed the specificity of the Pfeiffer test for differentiating the true *V. choleræ* from water vibrios. Hankin (1896[1 & 2]) has shown that the Ganges and Jumna river water kills *V. choleræ* rapidly, and this he attributes to a slightly acid reaction of these waters. D'Herelle (1922) explained the striking phenomenon of the bactericidal action of the waters of the Ganges and Jumna as due to the presence in the water of both these rivers of a specific cholera bacteriophage (see also work on cholera bacteriophage discussed on p. 360). Panyatatou (1913) found Nile water unsuitable for the life of the vibrio. Stamm (1914), at height of an epidemic, found non-agglutinating vibrios frequently in river water, but the agglutinogenic character was very constant, although variant colonies appeared amongst vibrios which had been living in water.

Tomb and Maitra (1927), in a recent paper, express the opinion that non-agglutinating intestinal vibrios constitute the reservoir of cholera both endemic and epidemic. The curve of cholera in Asansol Mining Settlement, Bengal, closely followed the curve of vibrionic content of surface water supplies. Cholera can only become epidemic in any locality during those periods of the year when owing to climatic conditions vibrios are capable of persisting or multiplying in the drinking-water supplies of that locality. This seems a simple and interesting solution of a complex problem. It would be of interest to know if vibrios survive or not in the tanks of areas where there has been no cholera for some years. Further, in this connection, Webster (1927) states there is no present evidence indicating that saprophytic micro-organisms of low virulence present in a community undergo an increase in pathogenicity, or mutate, become widespread, and excite epidemics. Also, Raghavachari (1927), in Madras, has obtained no definite evidence that atypical vibrios become pathogenic and produce cholera under certain conditions.

It is impossible at the present time to criticize the observations as much further work with full controls is required.

Sea Water.

Jacobsen (1910) found that the vibrio lived up to 47 days in sea water. Zammit (1913) twice isolated true *V. choleræ* from sea water. Takano, Ohtsubo and Inouye (1926) consider that the length of survival of *V. choleræ* in sea water has a special and important bearing on the spread

of cholera in Japan. The cholera vibrios carried into coasts and ports of Japan by ships, e.g. in the sea water of the ballast tanks, from infected foreign ports may cause the disease amongst fishermen and boatmen, who in turn pollute the sea water and spread the disease widely.

Colin (1915) found that water containing CO_2 under a pressure of 10 atmospheres killed the vibrio in 10 hours.

Food Materials.

Milk. Kitasato (1892) states that non-sterile milk at 22 to 25° C. gave a culture of cholera vibrio for 1 to 1½ days. In sterilized milk it lived for 3 weeks. Hesse (1893[2]) considers unheated cow's milk is not a good medium, nor is sour milk, the vibrio being killed in a few hours. Sterilized milk is a good medium. Rosenthal (1910) states that *V. choleræ* and *B. bulgaricus* cannot live together in sour milk ; the latter is a prophlyactic in cholera. Heinemann (1915) has noted vibriocidal effect of lactic acid in milk ; a concentration of 0·45 per cent. killed *V. choleræ*.

Butter. Laser (1891) records that the vibrio lived for about a week in butter.

Fish. Takano, Ohtsubo and Inouye (1926) note that fish is regarded as the most important article of food for the spread of cholera in Japan. At room temperature the vibrios smeared on fish-meat survived 3 to 4 days, and in the ice-chest 10 to 12 days. *V. choleræ* is recovered even although the fish is entirely decomposed. In fish fed with food containing *V. choleræ* the organism can be demonstrated in the intestines ; if such a fish is removed to fresh clean water the vibrios disappear in 4½ days.

Oysters and shell-fish. Remlinger and Nouri (1908) isolated cholera and other vibrios from oysters and mussels. Takano, Ohtsubo and Inouye (1926) state that when oysters, shelled or unshelled, are placed in fresh sea water or salt water contaminated with *V. choleræ* the vibrio enters the stomach in 1 minute. When oysters and clams are kept in cholera-polluted sea water at a temperature of 0 to 5° C., *V. choleræ* survives for 1½ months ; at 22° C. for 15 to 20 days. *V. choleræ* grows much better in the oyster than on ordinary fish. When smeared on the surface of oysters which are then soaked in 1 per cent. acetic acid, *V. choleræ* survives for 60 minutes, and in the stomach of the oyster for 7 hours : with 2 and 3 per cent. acetic acid on the surface, the vibrio survives for 15 and 7½ minutes respectively, and in the stomach for 7 and 2 hours : with 4 to 5 per cent. on the surface, the vibrio is killed immediately, but lives in the stomach for 45 minutes. So *V. choleræ* on surface of the oyster is easily destroyed, but not so easily in the digestive tract.

Coffee, tea, &c. Friedrich (1893) showed that in 6 per cent. coffee the vibrios were killed in 2 hours ; in 4 per cent. tea in 1 hour. In coffee and milk the vibrio lives 8 hours. In beer the vibrio can live only 3 hours, and, in wine, it is killed in a quarter of an hour.

Lal and Yacob (1926), in India, have investigated the relative suitability of certain foodstuffs as media for the cultivation of *V. choleræ*, with special reference to their relative role in the dissemination of cholera. Articles containing salt and animal or vegetable protein, e.g. meats and cooked *dal* (vetch) are particularly suitable. Chillies and onions—contrary to popular belief—do not inhibit *V. choleræ*. Fats form poor culture media, whilst sour articles are inhibitory.

Insects.

Flies. Guiseppi (1899) notes that *V. choleræ* can pass through the intestine of flies and remain living and virulent. Heiser (1908) finds the frequent reappearances of cholera in the Philippine Islands from 1902 to 1908 associated with flies, fish and water fleas. Observations by Graham-Smith (1910) and others support the view that the cholera organism can be carried in a virulent condition by flies, both externally and internally, for a significant length of time after they have fed upon or come in contact with infected material. Passek (1911) observed an alteration in virulence in the intestines of flies. It was present in highest degree 12 to 24 hours after ingestion, and disappeared in 72 hours. Virulence is increased by symbiosis with *B. proteus*. Eckert (1913) does not think flies are transmitting agents. Roberg (1915) notes that the spread of infection occurs by soiling of food by flies.

Cockroaches. Barber (1914) considers that cockroaches and ants act as carriers of *V. choleræ* for 48 hours. Toda (1923) found the vibrios in ship's cockroaches 1 to 3 days after feeding on fæces containing them.

Swallows.

Calderini (1913) found *V. choleræ* in migrating swallows.

Bank Notes.

Jettmar (1927) found that *V. choleræ*, when dried on bank notes, touched by fingers infected with cholera stool, remained alive up to 4 hours, and that the vibrio, cultivated from bank notes 4 hours after contamination, showed the same cultural and serological qualities as the original strain.

Effect of Physical and Chemical Agencies.

Drying : Temperature.

Borntrager (1892) records that *V. choleræ* is killed by drying in 2 hours, at 80° C. in a few minutes, and at 80 to 100° C. dry heat immediately. Kitasato (1892) found no difference in effect of drying on old and young cultures. Kasansky (1895) states that *V. choleræ* can be frozen and thawed several times without killing.

Irradiation.

Sunlight. Orsi (1907) found cultures of *V. choleræ* made from survivors of exposure to sunlight to be more virulent than original strain. Conor (1912) noted that actinic rays kill *V. choleræ* after some time.

Ultra-violet rays. Galeotti (1916) found that *V. choleræ* is killed in 1 minute by the quartz lamp 20 cm. distant (75 volt) in salt solution; in 30 minutes in blood-serum; in 2 hours in broth and urine; and in 2½ hours in milk. Photo-dynamic substances like fluorescin increase vibriocidal power. Schiavoni and Trerotoli (1913) report that a mercury vapour lamp, 75 volt, 20 cm. distant, with the temperature never above 20° C., kills *V. choleræ* in normal saline in 1 minute.

X-Rays. Rieder (1898) finds X-Rays have both an inhibitory and vibriocidal action.

Disinfectants.

Chlorine. Ditthorn (1915) noted that 0·28 mgm. of chlorine per litre of water, kills *V. choleræ* in 10 minutes, and that chlorine can sterilize the most impure water. Conor (1912) found that 2 mgm. chloride of lime per litre of water kills *V. choleræ* in canal water in 8 hours. Harding (1910) reports that chlorine 1 : 1,000,000 is sufficient for disinfecting water containing cholera vibrios: more is required when organic matter is present.

Soap. Jolles (1893) found that all soaps have some degree of vibriocidal power. Murillo (1912), however, found that soap is not vibriocidal.

Iodoform. Bujwid (1892) noted that the inhibiting and vibriocidal action of iodoform is very special and is not produced by camphor, naphthalin, turpentine, and thymol. Riedlin (1888) found that *V. choleræ* is strongly inhibited by iodoform and essential oils. It is interesting to note that the latter are used in the the treatment of cholera.

Lime. Giaxa (1890) reports that 20 per cent. milk of lime is vibriocidal.

Ozone. Schubert (1914) found that ozone completely sterilizes water.

Charcoal. One hundred c.cm. water to which has been added a loopful of *V. choleræ* and 1 gm. charcoal is sterile in 15 minutes.

Phenol. Koch and Gaffky (1887) found that phenol, 1 per cent., killed *V. choleræ* in 5 minutes; 0·5 per cent. in 10 minutes.

Mercury perchloride. In a dilution of 1 in 2 or 3 millions it killed the vibrio in 5 to 10 minutes.

Hydrochloric and sulphuric acids. 1 : 10,000 kill the vibrio in a few seconds.

Chloroform vapour is fatal to *V. choleræ.*

Aniline Dyes.

Shiga (1913) was able to increase the resistance or 'fastness' of the cholera vibrio 100-fold to the action of certain dyes. Signorelli (1912) showed that agar coloured with methyl green or azolitmin, is decolorized by the cholera vibrio, but the colonies do not take up the dye; on agar tinted with erythrosin, safranin, orcein, dahlia, the colonies are intensely coloured and the culture media are decolorized. The strongly coloured colonies rapidly lose their virulence for guinea-pigs.

Practical Diagnosis of Cholera.

By E. D. W. Greig.

Introductory.

The bacteriological diagnosis of cholera being the basis of preventive measures against the disease requires careful consideration. Both certainty and rapidity are essential. Before reaching a final decision, evidence of three kinds should be obtained and weighed, namely (1) clinical—this is often striking and sufficient; (2) epidemiological; (3) bacteriological. If all three are positive, then the case or the outbreak is true cholera; if (1) and (2) are positive the case or outbreak should be viewed with great suspicion, even although (3) may be negative. On the other hand, although the finding of the *V. choleræ* in, say, a water sample, in the absence of clinical or epidemiological manifestations is a matter of grave importance, yet before reaching a decision that cholera infection is in progress the whole circumstances must be investigated with great care.

Routine Procedures.

Material.

From patient. (1) Fæces. (2) Vomit. (3) Blood.

From corpse. (1) Clothing soiled with fæces. (2) Fæces from rectum. (3) Post-mortem. One or two coils of the ileum should be ligated at both ends and removed.

Water. About one litre is required from the surface in sterilized containers.

Smears on slides should be prepared from the fæces, vomit, &c. A peptone water tube and three agar tubes should be inoculated from the fæces.

Collection of Samples.

Fæces. Containers should be wide-mouthed glass cylinders with cork fitted with spoon. The glass cylinders should fit into a wooden box. Glass stoppered bottles may also be used. Careful packing is essential to avoid damage. All containers should be sterilized by boiling and no acid or disinfectant should be used. They should not be washed out with unsterilized water.

Vomit. This may be dealt with as above described.

Blood. Samples may be conveniently taken in Wright's capsules and sealed.

In cases where it is difficult to obtain fæces, e.g. in carriers, rectal swabs with sterilized wool may be taken and submitted for examination.

Transport.

If possible, all material should be sent packed in ice; with water this is essential in hot climates.

All the specimens should be *carefully labelled*, giving the following information: (1) name of patient; (2) sex; (3) caste, if any; (4) age;

(5) place of attack ; (6) if traveller, his home or starting-place ; (7) nature of attack ; (8) day and time of attack and death ; (9) date of taking sample ; (10) name and residence of Medical Officer to whom results of examination are to be communicated. The package should be marked ' Urgent '.

Examination of Material.

The morphological, cultural and biochemical characters of the cholera vibrio have already been dealt with in detail. In this section, a succinct résumé of the procedures is given for convenience.

Microscopical Examination.

Stained preparations.

Vibrio. Smears of a flake of mucus in the stool, which in a typical case has the well-known ' rice water ' character, are made on slides without much rubbing. The films are fixed by flaming, stained for about 1 to 2 minutes with *Ziehl* carbolic fuchsine solution diluted 1 : 5, and examined with a 1/12 in. oil immersion lens. If present, the characteristic vibrios will be seen, and a diagnosis may be made by an experienced worker after examination of such preparation. It has to be clearly remembered, however, that other vibrios occur in the stool—the so-called cholera-like vibrios.

Cellular elements in stool. In addition to examining for the presence or absence of the vibrio it is necessary to study the character of the cellular elements present, and from this useful information may be obtained. Maitra and Ganguli (1925) state that : ' The cells are usually the altered epithelial cells of the small intestine with badly staining nuclei and very little protoplasm round them '. This would be helpful, perhaps, in differentiating cholera from acute bacillary dysentery.

Hanging-drop preparations.

Particularly when vibrios are not numerous, a hanging-drop of peptone water may be made and a small portion of stool introduced into it with a platinum loop. It is placed in the incubator at 37° C. for 1 hour. The margin is then examined under 1/12 in. oil immersion lens. If cholera vibrios are present they are frequently seen in large numbers at the edge of the drop and are arranged like ' fish in a stream '. This arrangement is seen in the stained preparations also.

It is necessary to bear in mind that even in some acute cases of cholera very few vibrios may be found on microscopical examination, and, in some cases, none at all, although, subsequently, they may be successfully cultivated.

Fallacies.

In microscopical examination of stools, delicate spirilla are sometimes seen, both in normal and loose stools. They are less bent than the cholera vibrio and stain badly ; sometimes they are seen in pure culture in the mucous flake. They are not cultivable on the usual media employed.

Kasarnowskaya and Kritsch (1922) state the cholera vibrio tends to form rods in protein-free media, and in Russians, who are mainly vegetarians, *V. choleræ* takes the form of rods. Rod formation may, they think, cause mistakes in diagnosis. Rod formation of cholera vibrios in the stools of Indians, who are largely vegetarians, was not noted.

Cultural Methods. (See also p. 350).

Peptone water.

With a view to enrichment of *V. choleræ*, large loopfuls of fæcal matter are planted in 4 to 6 tubes of peptone water (pH 8·0 to 9·0), or 50 c.cm. flasks containing peptone water may be inoculated with ½ to 1 c.cm. suspected fæces. The tubes and flasks are incubated at 37° C. for 6 to 8 hours. The surface films removed with a platinum loop bent at right angles to wire are then examined by the hanging-drop method and preparations stained for *V. choleræ*, as described above. If no vibrios are seen, or very few, a second series of peptone water tubes or flasks is inoculated and incubated for a further period of 6 to 12 hours. A third series in some cases may be required. Stern (1915) uses fuchsine in the peptone water.

Cholera red reaction. This may be tested at this stage. The method is described on p. 362.

Other methods of enrichment.

Berka (1915) recommends peptonization of the stools to increase the number of vibrios. Ottolenghi (1911) uses a bile medium for enrichment and finds it rapid. Bandi (1910) recommends growing the stool in the presence of antiserum. He uses a drawn-out test tube, containing 5 c.cm. peptone solution ; to this a quantity of anticholera agglutinating serum is added. The test-tube is inoculated with one loopful of suspected fæces, and incubated at 37° C. If *V. choleræ* is present, at end of 2 to 7 hours numerous small agglutinated clumps of vibrios will fall to the lowest part of tube.

Agar plates, 3 per cent., reaction pH 8·6 to 9·6.

The plates are poured and carefully dried by placing slightly open in the incubator at 37° C. for half an hour. A flake from the stool picked up and washed in several changes of sterile normal saline, a loopful of fæcal matter suspended in peptone water or the fluid from the surface of the peptone water of the tubes or flasks, referred to above, is spread on the surface by means of a bent glass rod, or by a platinum loop, in successive strokes. Three such plates may be used. That the agar medium should be alkaline with the correct reaction is most important for the successful cultivation of *V. choleræ*. If typical cholera colonies are obtained on plates made direct from fæces or intestinal contents, the diagnosis is simpler than when plates are prepared from material which has been enriched in peptone water, as the latter forces the growth not only of the true cholera vibrio but also saprophytic vibrios. The agar plates are placed in the incubator at 37° C.

Gelatin plates.

Gelatin plates may also be used and the cholera vibrios have a characteristic appearance on them ; but in hot climates it is not possible to use gelatin, and so its applicability is much more limited than the agar plates which are very generally employed. McLaughlin (1909) states that in the United States of America the use of gelatin plates is no longer obligatory in the diagnosis of cholera.

Selective media for plating.

Dieudonné (1909) recommended a special blood alkali agar medium. The cholera vibrio grows better on this medium than on ordinary agar. The alkaline reaction restrains other organisms, but permits the vibrio to grow. It has the advantage that fæcal matter may be smeared directly on it. The plates are prepared and inseminated in the manner described above. The colonies of vibrios have a characteristic appearance ; by reflected light they are grey and clear like glass. Greig used this medium throughout his investigations on cholera in India and found that it gave very reliable results. Bürgers (1910) considers the medium so satisfactory in the diagnosis of cholera as to render the Pfeiffer test unnecessary. No alteration in agglutinability occurs, nor in form and staining reaction of the vibrio. Various modifications of Dieudonné medium by Pilon, Kabéshima, Esch, and others, are described on p. 357, as well as other special selective media.

Identification of *V. choleræ.*

Single colonies are isolated from the incubated plates and subcultures made, for the study of :

(a) *Morphological characters* (p. 346).

(b) *Cultural characters* (p. 352).

Variation has been noted by numerous observers and has introduced considerable difficulty into the bacteriological diagnosis of cholera. This subject has been already discussed fully.

(c) *Serological tests.*

These are the most important for the specific diagnosis of cholera vibrio. They have been already described. They are merely enumerated here : (1) Agglutination (p. 367, 377) ; (2) Agglutinogenic capacity (p. 378) ; (3) Pfeiffer's test (p. 370) ; (4) Absorption tests (p. 369) ; (5) Complement-fixation test (p. 371) ; (6) Opsonic index (p. 371).

(d) *Biochemical tests.*

These have been already dealt with.

(1) *Hæmolytic tests* (p. 364) ;

(2) *Precipitation of Vibrio protein by concentrated salt solution.*

Porges (1906) carried out a research on the relation between bacterial

agglutination and precipitation phenomenon of colloid. Greig (1913[4]) examined on a large scale the question of precipitation of vibrio protein by concentrated salt solution and its relation to the bacteriological diagnosis of cholera. His results were as follows : 178 cultures of cholera vibrio were investigated and of these 164 were salted out completely, and 12 in traces or incompletely even in 90 per cent. concentration. Strains difficult to salt out reacted, however, with specific cholera serum to titre limit.

Of 41 cultures of cholera-like vibrios studied, 27 were not salted out at all, 8 were salted out in traces, 6 were salted out completely in 90 per cent. magnesium sulphate. The strains which salted out easily were, like other cholera-like vibrios, not agglutinated by a cholera serum.

It will be seen that there is not a complete agreement in the phenomenon of agglutination and salting out of bacterial protein, but there is a marked difference in regard to salting out of bacterial protein between the true cholera and cholera-like vibrios. Liefmann (1913) inclines to the view that strains which are not capable of being salted out must occupy a special position.

(e) *Animal experiment.*

(1) *Intraperitoneal injection of guinea-pigs.*
(2) *Intramuscular injection of pigeons.*

Both methods are largely employed in the bacteriological diagnosis of cholera. Intraperitoneal injection of small quantities of an agar culture of cholera vibrio produces a fatal peritonitis in guinea-pigs. The cholera vibrio compared to other vibrios is, as a rule, feebly pathogenic on intramuscular injection of pigeons and this is used as an additional method for differentiating the two. Suzuki (1926) studied vibrio ' kadikjo ', a member of the El Tor group, by animal inoculation. The question of its inclusion in the group of 'semi-parasites' is considered. It differs markedly from the majority in the ease with which infection is produced in the guinea-pigs. It may be considered to stand high in the list of ' semi-parasites '. See also (p. 424) where this organism has been discussed further.

(3) *Protection by means of living vibrios.* Three doses corresponding to, say, 1/60, 1/30, 1/15 of an agar slope of cholera vibrio are given subcutaneously to guinea-pigs. Then 10 days after the last immunizing dose, 1/20 of an agar culture of the unknown vibrio is injected intraperitoneally. The protection given is noted.

Examination of the Patient's Blood.

This is important in the bacteriological diagnosis of cholera. It is done to determine the presence or absence of specific agglutinins and bacteriolysins.

Agglutinins.

Greig (1915[2]) studied fully the agglutinins in the blood of cholera cases. The result of investigation may be summarized as follows :

1. *Agglutinins in blood of fatal cases of cholera.*
   Absent in the majority of cases.
2. *Agglutinins in blood of non-fatal cases.*
   Examination from first to forty-fifth day.

(a) *Development of specific agglutinins in blood of cholera cases begins early,* in some cases, second day of disease ; reaches its maximum about the sixth day, and remains high to the seventeenth day. A drop appears to occur about the twentieth day. The titre varies from 1 in 40 to 1 in 1,000 dilutions.

(b) *Agglutinins in relation to the diagnosis of cholera.* Agglutinins are not marked till about the sixth day ; the test at this stage is a valuable practical aid in the diagnosis, as for example, in persons arriving by sea who may have suffered from a suspicious illness on the voyage.

(c) *Agglutinins in relation to detection of cholera carriers.* This is specially useful in the convalescent ' carrier '. If a distinct agglutination reaction is obtained on blood examination, a careful investigation of the stool should be made.

(d) *Agglutinins in the blood of cholera cases in which true cholera vibrio and a cholera-like vibrio were cultivated from the stool.* Agglutinins were developed only against *V. choleræ* and never against a cholera-like vibrio.

(e) *Agglutinins in the blood of cholera cases in which cholera-like vibrios only were cultivated from the stool.* In none of the cases did they agglutinate the cholera-like vibrio isolated from the stool, but they did agglutinate the standard *V. choleræ.* In this group the detection of specific agglutinins may be a valuable aid in diagnosis.

(f) *Agglutinins in the blood of cases in which neither the cholera nor cholera-like vibrio were cultivated from the stool.* In a few of these cases the sera did agglutinate the standard *V. choleræ.* By this test the diagnosis, which otherwise might be missed entirely, may be established.

Bacteriolysins.

Bacteriolysins may be tested by the Pfeiffer test or *in vitro* (p. 370).

CARRIERS.

As has been shown, the discharge of vibrios in the stools of ' carriers ' is intermittent. Hence repeated examination of the stools is necessary for the detection and diagnosis of ' carriers ' ; at least three times every second day, preferably more frequently. The methods of examination have been described on p. 403.

The International Conference, Paris (1911) recommended that in practice all cases of gastro-enteritis in which vibrios resembling the cholera vibrio are found which agglutinate 1 : 100 with high titre serum (1 : 4,000),

or give a positive Pfeiffer reaction, should be regarded as cases of cholera. Carriers, they report, are numerous in the neighbourhood of cases, but otherwise few. At German stations on Russian border, there were 3 out of 5,200 persons examined ; at Egyptian stations, 22 in 15,000. The excretion by a carrier is short—2 to 3 weeks—exceptionally 12 months. Pontano (1912) finds the cholera vibrio of carriers identical in all respects with *V. choleræ* in cases of cholera. Ravenna (1911–12) finds also the virulence identical.

The ' carrier ' problem in cholera is discussed also on p. 394.

Examination of Water.

For the diagnosis of cholera, it may be necessary to make a bacteriological examination of water. At least one litre of water should be collected, and, if it has to be despatched to a laboratory, the instructions given at the beginning of this section for the transport of specimens, &c., should be observed. The method of examination has been described on p. 360.

El Tor and Other Cholera-like Vibrios.

This group has caused considerable difficulty in connection with the bacteriological diagnosis of cholera. They are considered fully by themselves on p. 424.

Immunization.

By W. F. Harvey.

A special importance attaches in cholera to methods of prophylactic immunization for countries which are free of the disease and desirous of guarding against its entry. It is likewise a matter of great importance for bodies of men travelling into districts and countries in which the disease is rife or endemic. The immunity of countries from cholera is probably much more closely related to the amount of traffic with other affected countries, and the precautions taken at the frontiers or on first occurrence of the disease than to soil or climate. So also the relative immunity of individuals, of races and of nationalities finds its most probable explanation in the simple facts of physique, habits, mode of life and hygiene.

Natural and Acquired Immunity in Man.

There is little reason to suppose that any natural specific resistance to cholera is found in man. Acidity of the gastric juice has always been regarded as one of the greatest barriers to the passage of the cholera vibrio into the intestine : the vibrio is rapidly killed off by the action of the hydrochloric acid of the stomach and it is highly probable that this, rather than natural immunity, is the chief reason for failure to infect after ingestion. Much of the experimental work on animals points in this direction likewise. Very young animals in which the hydrochloric acid

of the stomach is absent or has not reached any degree of concentration can be infected, as also can older animals after neutralization of the acid with sodium bicarbonate. Other factors, in the very young animal, may be the absence of intestinal bacteria and the absence of complement from the blood. In the intestine the mucus coating the surface forms a barrier the removal of which facilitates systemic infection, as is shown by the action of ox bile in favouring that infection. Part of the inability to bring about infection may be attributed to the antagonism of the normal intestinal bacteria to the growth of the cholera vibrio.

Natural immunity likewise must be ascribed to the natural modes of resistance, cellular, phagocytic, or humoral, possessed by the body to the invasion of any pathogenic organism. As cholera is not, in its essence, septicæmic in character, the weight of evidence points to the chief factor in resistance, after the gastric juice, being the intestinal surface. But such natural immunity is wholly non-specific, however much it can be artificially increased so as to become specific. An artificial non-specific immunity to intraperitoneal infection with cholera vibrios can be demonstrated in animals such as guinea-pigs. They can be protected against a lethal dose by previous injection of organisms which are in no way related to the cholera vibrio. Such organisms as *B. coli*, proteolytic organisms such as *B. proteus*, *B. pyocyaneus* and *B. prodigiosus*, or even non-organismal material such as nutrient bouillon, nucleic acid, tuberculin, acridin preparations, &c., can effect a certain amount of protection. This protection, however, differentiates itself from specific immunity by its early appearance and its early disappearance : it may appear already on the day after injection (Pfeiffer and Issaeff, 1894). The injection also of non-specific organisms does not give rise to an antiserum to the cholera vibrio. A specific antiserum is developed only at a definite interval after injection. Specific agglutinins are not evident until after the fifth or sixth day, and protective power of such a specific serum is demonstrable for a considerable time after the injections have ceased, whereas little protection is evident with non-specific inoculation in animals after the tenth day and it has disappeared by the fifteenth.

Any natural immunity, then, possessed by man to cholera, may be ascribed to circumstance and to non-specific agency. Artificial immunity is of two kinds. There is the immunity conferred by an attack of the disease and the immunity due to artificial inoculation. An attack of cholera, it is stated, confers a high degree of immunity against a second attack. The actual evidence for this statement is not very easy to find. The probabilities against an individual being in a position to contract a second attack of cholera must be great. This does not apply merely to his being in contact with cases of cholera, but to the likelihood of any individual's contracting infection even after the ingestion of the cholera vibrio. The possibility also that the individuals of a community in which the disease is endemic may suffer from slight attacks which do not attract notice, and thus become immunized, must be held to be probable. The

case of diphtheria points significantly to this possibility. That naturally acquired immunity does result seems certain, but little information exists as to the longer or shorter duration of that immunity.

Active Artificial Immunization of Animals.

The animal infection on which most of the attempts at artificial immunization have been tested is the peritonitis set up by injection of a considerable dose of virulent cholera vibrios in the guinea-pig. Such a peritonitis can be set up by other organisms. A specific cholera immunization will nevertheless protect against a cholera peritonitis to a degree and for a length of time which is not obtained in animals immunized with other organisms, and will not protect to the same degree against peritonitis of other causation. The test is carried out either by mixing a suspension of virulent cholera vibrios in definite proportions with the serum of the immunized animal or by injection of the vibrios alone in an immunized animal. But much of the evidence of active artificial immunization of animals to cholera is based upon the serum response consequent upon injection of cholera vibrios. These may be either living or dead and the content of the serum of the treated animal in antibodies is taken as the measure of the response. By all the usual methods of injection in animals, subcutaneous, intraperitoneal and intravenous, it is particularly easy to obtain sera with high content in agglutinins, bacteriolysins and antibodies. The titre of the serum is of a high order, and there is no apparent difference in the character of the antibodies obtained by injection of dead cholera vibrios in place of living vibrios. These antibodies can likewise be obtained by the use of bacteria-free cholera extracts. The antibodies thus obtained are undoubtedly specifically antibacterial, that is to say, they act essentially upon the bacterial protoplasm, killing the vibrio, agglutinating it, precipitating extracts or sensitizing the organisms to ingestion by leucocytes. The systemic effects of a cholera infection are, however, regarded as an intoxication and the question arises whether the artificial immunization of animals is also antitoxic in character. The standard cholera vibrio does not produce an exotoxin in fluid media and the question of antitoxic immunity is reduced in such case to one of anti-endotoxic immunity. It has been found difficult to neutralize the products of disintegration of the cholera vibrio by means of an antiserum and to protect animals by active immunization against the lethal effect of such products.

In recent years the question of oral immunization has received much attention and much experimentation has been carried out upon animals on this subject. Generally speaking, all attempts at immunization of animals by oral administration of cholera vibrios have been unsuccessful. Besredka, however, has evolved a method through which he claims to have been able to immunize animals by causing them to ingest ox bile as a preliminary to a subsequent dosage with cholera ; by this method there is produced a local immunity of the intestinal wall. The bile denudes the intestinal wall of mucus and so affects it that the cholera antigen is

able to act upon it and bring about a state of local resistance to attack. The completely immunized animal shows no antibodies in the serum, although there may be a temporary appearance of agglutinins in the blood with the administration of the first dose. These disappear altogether with further administration of antigen and yet the immunization continues to increase. The absence of antibodies is claimed to speak for the immunity being local and not general. Bile has been chiefly used for the sensitization of the intestinal wall, but other substances such as dysentery toxin, podophyllin, and enterolytic serum, serve the same purpose.

The repetition of the work of Besredka has furnished very contradictory results. The bile, which is administered for sensitization of the intestinal wall to antigen, injures the epithelium of the mucous membrane, and the regulation of the dosage to give sensitization without serious damage is not at all a simple matter. There is also danger that intestinal bacteria may be enabled to penetrate into the body. Very large doses of living cholera vibrios are required for oral immunization and the killed cholera is not so satisfactory for the purpose, even though the immunizing antigen be preceded by bile. The method may not, in reality, be a purely local immunization, for the bile, by denuding the intestinal wall of epithelium, allows the vibrios entrance into the tissues over a large extent of surface, whereby the same general immunity is brought about as if the antigen had been injected parenterally. The absence of antibodies is not a complete proof of the absence of a general blood immunity: that immunity even in the absence of antibodies may still be a general cellular immunity. The active immunity obtained by bile vaccine, orally administered in the rabbit, has also served to protect the animal against intravenous infection with cholera vibrios, but not against intraperitoneal infection. From the many contradictions which this method of immunization has raised, it may be concluded that further work on the subject is required before judgment can be pronounced.

The oral administration of potent bacteriophage in cholera (D'Herelle and Malone, 1927) is not so much a method of immunization as of treatment. It is still too early to say very much about it; it has, however, been tried in man.

Analysis of Immunity Mechanism in Animals and Man.

Analysis of the immunity mechanism of cholera turns very largely on the view held with regard to the nature of the toxic product of the cholera vibrio, which by its absorption from the intestine gives rise to the symptoms of the disease. The two types of toxin with which we have to deal in pathogenic micro-organisms are the endotoxin and the exotoxin.

The researches of Pfeiffer on the standard cholera vibrio are in favour of the endotoxic character of its toxin. He extends the idea further to a denial of the existence of any antitoxic immunity response upon the part of the invaded organism to the cholera endotoxin, that is, even to

the products of disintegration of the protoplasm of dead cholera vibrios. Pfeiffer, however, does not deny the existence of a cholera immunity. He maintains that immunity to cholera consists in the ability of the immunized animal to kill off the cholera vibrio before it has been able to increase to such numbers as will give rise on the death of the vibrios to an amount of toxin sufficient to produce death. In suckling and young animals in which a form of intestinal cholera infection has been produced, the absence of inhibiting intestinal organisms and possibly the absence of the non-specific serum factor (complement of Ehrlich) would account for the multiplication of the cholera vibrio in these cases to the extent required to produce death. The neutralization of the acid of the gastric juice by alkali and the paralysis of the intestine by means of opium, which were the methods used by Koch to produce an intestinal infection, are factors of like nature, according to this hypothesis. Various other explanations of cholera immunity and cholera infection are put forward, all of which are based on the various general theories of immunity which have been propounded.

In cholera we have what are called the cholera-like organisms, some of which are decidedly toxic in their action and produce a definite soluble hæmotoxin in culture media. They are exemplified by the El Tor vibrios and the vibrios called ' paracholera ' generally by Kraus, Castellani, Mackie and others. The cholera-like organisms may be identical serologically with the standard cholera organism and yet produce a characteristic hæmotoxin, or they may be serologically differentiated and produce hæmotoxin, as in the case of the paracholera races of Mackie. It is maintained by Kraus, Kraus and Kovács (1928) that an antitoxin which they affirm can be obtained to the toxin of the El Tor vibrio, will act not only upon the toxin of the homologous organism, but also upon that of the standard cholera vibrio.

A recent development of immunity theory which applies very particularly to the case of cholera is the theory of local tissue immunity. This theory is itself dependent on the theory of a selective action in infection of various bacteria with respect to certain tissues. Some are dermotropic, some neurotropic, and others, such as the cholera vibrio and the dysentery bacillus, enterotropic. Cholera vibrios in their attack on the organism are attracted towards the tissues of the intestinal wall. Whether they are inoculated intravenously, intraperitoneally or even subcutaneously, they are found (Masaki, 1922) in great part in the intestine. It is when the intestinal wall is injured, as occurs in natural cholera infection, that the cholera toxin is enabled to pass the barrier which the intestinal wall presents to its passage into the body. It may thereby produce the symptoms of cholera or it may stimulate the receptive cells to a heightened resistance. It is this increased resistance, when it occurs, that constitutes, according to this doctrine, a true immunity to natural cholera infection : it is a local cellular, not a general immunity, and is not accompanied by the production of antibodies. If any antibody is formed as a result of the

first passage of cholera endotoxin it disappears with the increase of the immunity, or, in other terms, with the heightening of the local resistance to absorption of toxic or antigenic products.

The ascription of immunity to a local tissue resistance turns very largely on the finding that serum immune bodies are absent in the animal, which has, as it is claimed, been rendered immune by the heightening of the resistance of the particular tissue liable to attack by the organism in question. This deduction, however, is scarcely warrantable. Animals may be parenterally immunized and may still be resistant even after the antibodies in the blood have disappeared, as has been shown by Hedley Wright (1927) for pneumococcus. Moreover, it is not at all certain that general immunization, with antibody formation, does not occur after the administration of bile cholera vaccine. The bile injures the intestinal surface, which allows of the passage of cholera vibrios or their unchanged products, and there is thus produced an ordinary type of systemic immunization in precisely the same way as by parenteral injections of antigen. So at least it is argued by opponents (Klüchin and Vigodtschikoff, 1925; Otten and Kirschner, 1927) of the Besredka theory. Again it is argued that the fatal result from ingestion of cholera vibrios after the previous administration of bile is not due to the cholera vibrio, but to an activation of intestinal bacteria, especially *B. coli*, by which they are enabled to penetrate the injured intestinal wall and to produce death. This result can be prevented by previous *parenteral* immunization of the test animal with *B. coli* vaccine.

Types of Immunization.

Living vibrios. The first essays at immunization were chiefly those which used living vibrios and are associated especially with the names of Ferran and of Haffkine. They have now been practically abandoned in favour of immunization with dead vibrios.

Dead vibrios. Gamaléia (1888[2]) was the first to show that vibrios killed by heat could take the place in immunization of the living organisms. The application to man of the now generally used vaccine of killed organisms was due largely to the work of Wright and of Kolle. The main contention in favour of the employment of dead organisms has been that the antibodies developed from their injection differ in no respect from those obtained with living organisms. Recent researches into the nature of antigens have shown that at least two types of antigens exist in many organisms, including cholera: they may be differentiated by means of their respective degrees of thermo-lability. In the case of cholera, which is mono-flagellate, the heat-labile antigen is naturally very deficient. It is the heat-stable antigen (somatic antigen, resisting a temperature of 100° C. for 1 hour) which gives protection in animals against intraperitoneal lethal doses (Fairbrother, 1928). Much controversy has gone on regarding the use of a highly virulent or an avirulent strain of vibrio for purposes of active immunization. The most recent research points to the necessity of

reconsidering this question from a new point of view. Organisms of rough colony or R type are usually avirulent and do not produce antibodies to the pure smooth colony or S type of organism. Although this has not been definitely established, it is probably true for the cholera vibrio. As the type of cholera vibrio which is avirulent for animals is likely to be avirulent for man, the inference is that this type should be excluded from preparations intended for active immunization. It seems probable that specially selected types of vibrio, which have proved effective in active immunization, will be the desirable types to use for this purpose.

Bacterial extracts. Many attempts have been made to extract from bacteria the antigen or immunizing substance which is concerned with the development of immunity to infection. If such a body can be isolated in a reasonably pure state, the possibilities of concentration and the accurate measurement of dosage become greatly enhanced. Comparatively little progress, however, has been made in this direction. Most of the researches have had for their object the separation of a cholera nucleoproteid with antigenic properties. Blell (1906) subjected cholera vibrios to the action of 1 per cent. caustic potash and precipitated nucleoproteids or allied bodies from the solution by acidification with acetic acid. This precipitate was successfully used for immunization of animals and the resultant serum was also used to save animals intraperitoneally injected with living cholera vibrios, when administered 1 to 4 hours after the infection. Besredka and Golovanoff (1923) prepared cholera ' antivirus ' by incubation of a bouillon culture for 8 to 10 days and filtering. The filtrate was resown with the cholera organism and refiltered after a second incubation of 8 to 10 days. They obtained some protection in guinea-pigs with the filtrate. Most of the work in this direction has not progressed much further than the stage of animal experiment.

Toxin. Active immunization of animals with toxin has been mostly used in animals in the hope of obtaining from them a serum which would be active in man against the effect of cholera toxin. The toxin used for the purpose has varied from one which is frankly an endotoxin or product of disintegration of bacterial bodies to a supposed true exotoxin, usually derived from one of the highly toxic vibrios such as the El Tor, which are closely related to the standard cholera vibrio. Animals can be gradually immunized to the lethal effect of a bacterial filtrate of a bouillon culture of cholera or one of its allies and so far may be said to be immune to cholera toxin. The sera obtained by such a process of immunization, in the larger animals such as the horse and the donkey, have been used therapeutically. All these methods must depend ultimately, so far as they are intended to be used in man, on the results which statistical analysis shows has been obtained by their employment. But a great deal of our knowledge of the possibilities which the several preparations offer of practical utility has been built up from research on the serum antibodies which are developed on their injection into animals. The work on this subject of Pfeiffer and Wassermann (1893) was elaborate and fundamental. This

work, with much which followed it, was laid upon the foundations of a thoroughgoing belief in a humoral theory of immunity, and, although some of that theory requires modification at the present day, it is unlikely that it will be omitted altogether from any more complete theory of the subject.

Modes of Administration of Immunizing Substances.

All the usual modes of active immunization with the cholera vibrio have been experimented with in animals—cutaneous, subcutaneous, intraperitoneal, intravenous, nasal, oral and intratracheal. The subcutaneous method is that which has, following animal trials, been chiefly used in man. In the production of sera for diagnostic use the intravenous injection of a suspension of killed vibrios is the method of choice and the rabbit the animal of choice. By the intravenous method the vibrios are distributed all over the body and the production of antibodies is rapid and the titre obtained 1 in 4,000 to 1 in 8,000 (Chick, 1916) or higher.

At the present time hopes are entertained that oral administration, especially under sensitization by bile, may serve to immunize man against attack during an epidemic, but the results so far obtained are not yet sufficiently convincing to lead to its general use. Intraperitoneal immunization is used only in animals and has a place intermediate between subcutaneous and intravenous as regards rapidity of production of antibodies. The common method is to give increasing doses of antigen intravenously at intervals of about a week, whereby, after 4 or 5 such doses, a high-titre serum is obtainable. The animal is bled about a week after the last dose. Immunization in man usually consists of two, sometimes three, subcutaneous doses at intervals of 7 or 8 days. In the case of the two-dose immunization, the second dose is double the first. The artificial immunization of man, on account of its high importance to the community, requires special notice.

Artificial Immunization in Man.

It is not difficult to immunize animals against experimental cholera infection, and, therefore, it might be supposed that cholera in man would be a disease which could be easily protected against. There is this great difference, however, between animals and man. Animals never acquire the disease in nature and the experimental infection in animals often bears scarcely any resemblance to the disease in man. Such is the case, for example, for the peritoneal infection so commonly used for demonstrating immunization efficiency or protection in animals. Active parenteral immunization again, in animals and in man, gives rise to antibodies circulating in the blood, and these antibodies have a powerfully bactericidal effect on cholera vibrios. But the disease in man manifests itself by the presence of cholera vibrios in the intestine where they cannot be affected by antibodies present in the blood-stream, and if we accept the view that the immunity produced by vaccines administered parenterally is not antitoxic, in any sense of the word toxin, then little

encouragement is afforded on such grounds for the administration of prophylactic vaccines by inoculation. This argument, however, is based solely upon the contention as to absence of any anti-endotoxic immunization, and on the view that the symptoms of cholera are due to the possibility of unchecked multiplication of vibrios in the intestine with absorption of the toxic products resulting from their disintegration.

In recent years the theory that the symptomatology and pathology of many diseases is due to selection of special tissues for attack by organisms (histotropism) has been vigorously applied to the case of cholera. The cholera vibrio on this theory is regarded as an enterotropic organism giving rise to a localized infection of the intestine ; its symptoms as due to the breaking down of the barrier presented by the cells of the wall of the intestine to the passage of cholera toxin ; immunity as due to a heightening of ability of the cells of the intestinal mucous membrane to present a barrier to the passage of the toxin. On this basis protection against cholera would be afforded by the vibriocidal character of the gastric juice, the antagonism of the ordinary intestinal bacteria to the growth of the cholera vibrio and the resisting power of the intestinal mucous membrane, natural or artificial, to the attack of the vibrio and passage of its toxin. The local immunity of the intestinal mucous membrane might be increased by parenteral methods of administration of cholera immunizing substance ; but the generally advanced contention is that, as the resistance of the intestinal mucous membrane in the natural infection is broken down by attack from the side of the intestine, so will that resistance which is developed by the same method be best calculated to protect. This, as we have seen, is the rationale of the use of bile with vaccine administered orally. The bile sensitizes, or it may be even to some extent causes breach in the intestinal surface whereby a small amount of toxin may be absorbed and the cells of the mucous membrane gradually rendered resistant. There are other aspects of the theory of cholera infection which might allow of the possibility of a general or blood immunity coming into play as well as a local intestinal tissue immunity. The undoubted enterotropism of the cholera vibrio has been invoked to explain the presence of the organism in the intestine without passing the gauntlet of the gastric juice : it is said to penetrate the surface of the body in the pharyngeal region, and to be conveyed by the blood to its selective site in the intestine. On such a basis general immunization would be effective against the vibrio in the course of passage to the intestine.

Prophylactic Vaccination.

The methods which involve the use of living or dead, virulent or avirulent organisms, bacteria, bacterial extracts or bacterial products are not so much opposing methods as methods differing in degree of efficiency, convenience or safety. The parenteral methods were the first employed. Kolle's vaccine is a suspension of the growth of a 24-hour agar culture of a standard cholera vibrio in 0·85 per cent. salt solution,

which has been killed at 56° C. and has 0·5 per cent. phenol added as preservative. Phenol alone may be used, as has been done in India, for killing the vibrios, or they may be specially treated for reduction of the toxic effect without affecting immunogenic power, as by ether to remove lipoids (Vincent, 1915) or by formaldehyde in the anatoxin procedure of Ramon. A sensitized vaccine, that is to say, one of which the vibrios have been treated with antiserum, has been used on a rather large scale in Japan. A suspension of such sensitized vibrios produces little or no reaction on subcutaneous injection and immunity is rapidly established. The rapid establishment of immunity for a population in which cholera has made an appearance is obviously a matter of great importance. The duration of the immunity by this method is, however, seemingly short. This is what might be expected from such a combination of active and passive immunization procedures. It is a moot question whether one is not simply dealing here with an active immunization by very small doses, either a residuum of unneutralized antigen or antigen liberated in small amounts from its union with antiserum.

The method of nucleoproteid immunization of Lustig and Galeotti has not made much headway in practice. That of the bacteriophage lysate of d'Herelle has still to prove its advantage over the older vaccines consisting of a suspension of organisms. The material alteration of the chemical constitution of the vibrio has to be kept in mind in the case of all proposals to use as immunizing substance the dissolved or the chemically treated organism. Moreover, the greater the complication that is introduced in the preparation of a vaccine, the greater may be the danger of the introduction of unwanted contamination. Such contamination may represent only a very remote danger, but has to be seriously considered when large-scale vaccination is embarked on. More important still may be the consideration which has come to the front in recent investigations of the precise type of antigen which should be used in the preparation of vaccines. The use simply of a large number of strains of cholera in a vaccine on the hit or miss principle has nothing to commend it scientifically. It is probable that the use of a recently isolated organism from a typical human case is advisable from all points of view. But if we take into account double and single antigenic characters and the specific immunizing properties of each antigen in the different types of true cholera vibrio, it may be necessary to standardize vaccines still further in regard to their component strains of vibrio.

A cutaneous method of parenteral cholera immunization has been reported on by Ciuca and Balteanu (1924) as giving rise to a strong immunity which is of the strictly cellular description, that is to say, unaccompanied by the production of serum antibodies. Although it has been shown that living cholera vibrios may be inoculated with safety, intravenously in man (Nicolle, Conor and Conseil, 1912) and are immunizing, there does not seem to be any good reason for the substitution of such a method of administration for the ordinary subcutaneous method.

There remains for comment the oral or enteral method of immunization. The theoretical aspects of enteral immunization have already been dealt with. A vaccine which can simply be taken by the mouth has much to commend itself from the point of view of convenience and ease of applicability on a large scale. The complication of the oral procedure is the necessity of giving bile as a sensitizing agent before the vaccine, the danger of injury to the gastro-intestinal mucous membrane by the bile, and its consequent liability to invasion by intestinal organisms, the large doses required and the difficulty of regulating dosage, and possibly also the necessity to use living instead of dead cholera vibrios in the vaccine.

Methods.

Many variations in the preparation of cholera vaccine exist, into the details of which it is not necessary to enter, as they follow the lines of variation of vaccines in general. It is desirable to select as far as possible strains which give no excessive reaction and which at the same time have good antigenic or protective quality. The symptoms which may follow cholera vaccination are as a rule quite slight and should not interfere with the individual's capability for work or, at all events, not for more than 24 hours. The type of symptoms which may be met with, although more often than not completely absent, are slight local pain and swelling, with similar affection of the corresponding glands, and general symptoms such as slight increase of temperature, seldom to more than 102° F., with headache. In a few cases when large numbers are being inoculated there may be some diarrhœa, vomiting, polyuria and even slight muscular cramps. The symptoms, if any, show themselves 3 or 4 hours after inoculation, reach their height in 6 to 8 hours and disappear altogether in 24 to 48 hours. In very exceptional cases anuria instead of polyuria and lowering of temperature instead of rise, have been observed. Great variation is seen in the symptoms following the second dose, and it may be said that these are sometimes less and sometimes more severe than after the first. A rash has been occasionally recorded after cholera inoculation, but it is doubtful whether this has any specific connection with cholera vaccine.

With large scale inoculation it is not infrequency of occurrence of troublesome sequelæ which impresses the individuals undergoing inoculation, but marked symptoms in any single individual. It is, therefore, important to keep in mind contra-indications to inoculation especially in large scale operations. And first there is the possibility of precipitating the occurrence of cholera in individuals who are merely healthy carriers for the time being. There does not, however, seem to be any sufficient evidence for an occurrence of this sort and there is no doubt that carriers in considerable numbers have been inoculated without the slightest disturbance. They react to the vaccine exactly as non-carriers do. It has been surmised too that cholera inoculation may tend to bring about an increase in the number of carriers, but of this again there is little

substantial evidence. It is well to avoid inoculation of individuals who are enfeebled from any reason and perhaps also those suffering from renal insufficiency. Almost all observers are at one in thinking that any negative phase condition which may exist, may be neglected even in the presence of an epidemic.

The blood-changes in subjects of inoculation have been followed out by Balteano and Lupu (1914). The agglutination in normal individuals does not usually transcend the 1 in 10 limit. With a two-dose inoculation it reaches a maximum, in about 3 weeks, of about 1 in 150, remains at that level for about 1 week, and then begins to decrease. The agglutination titre even after 3 or 4 months should be about 1 in 40, and after 5 months about 1 in 20 to 30. Bacteriolysins appear about the fourth day and their titre by an *in vitro* test may reach 1 in 150 and continue at such a level for 3 months. Obviously these are only indications of the levels to be expected, as they vary with number of doses used in inoculation and strength of vaccines, while individuals will show considerable variations from average values. They help, however, in giving some indication as to how soon a vaccine may be expected to immunize, a most important matter in the presence of epidemic cholera conditions.

Results.

The statistics are very difficult to interpret and it has to be admitted that no convincing data of the efficacy of vaccine in preventing cholera exist. Still less information exists on the duration of the efficacy. The difficulty centres mainly in obtaining two populations of the treated and the untreated which are with any certainty comparable. The totals of the inoculated are usually fairly accurately known, but those of the uninoculated are apt to be ill-defined and often only obtained by calculation. Without accurate knowledge of both these totals, comparisons of percentage morbidity or mortality are always open to grave doubt. It is the common practice, moreover, to publish supposedly favourable statistics and to find reasons for the unfavourable, if they be published at all. Selection in the statistical sense overshadows all the published data. It is little use comparing military statistics with civilian statistics or statistics where the inoculations are submitted to voluntarily. The age, the social standing, sex, physical fitness and the estimated degree of attached risk is seldom worked out as a frequency distribution for the treated and the untreated populations. Inoculations for cholera are often undertaken when an epidemic is in full swing, which is often very near to saying that those who undergo inoculation are but little at risk. Weaklings and ailing persons may not be inoculated as being unfitted for inoculation and yet come to be included among the untreated. Under such circumstances, the vaccination procedure is applied to a population which would not in any case suffer much from cholera, and yet the result will be claimed as a success for prophylactic vaccination on the basis of comparison of the mortality among treated and untreated. Modes of

collection of data of the treated and untreated may differ widely, and it is often the cases of illness and death among the untreated which are specially reported. It may be also that cases of attack and of death occurring amongst persons found when attacked to have been untreated, are included in the percentage rates, although they may not have been in the original totals of untreated. Such practices as these last would result in serious overweighting of the untreated population with morbidity and mortality.

We are thus, in the case of cholera, forced back upon analogy with other diseases in which vaccination has proved effective and upon animal experiment. Unfortunately for the evidence of animal experiment, the laboratory animal is not ordinarily capable of infection with true natural cholera. It is obvious then that what is required is the collection of statistical data on the strictly alternate case basis for the proof of efficacy of cholera vaccine.

We may refer here to some of the statistics which have been published on the results of cholera vaccination. Of the older results those obtained by Haffkine in India are the most often quoted, as those of Ferran are not very clear in detail. But these show surprisingly slight results. Take, for example, the result for one regiment (Haffkine, 1895) of morbidity and case mortality among the inoculated of 13·53 per cent. and 66 per cent. as against 18·75 per cent. and 72 per cent. in the uninoculated. Of course, such points as size of dose used and lapse of time since the protective inoculation had been carried out have to be taken into account. Powell (1899) gives for approximately equal numbers of inoculated and uninoculated, 2,941 and 2,235, the morbidity and case mortality 0·6 and 58 per cent. as against 2·3 per cent. and 69 per cent. Babes (1914) makes the comment on the effect of cholera inoculation in the district of Hiogo in Japan that it did not prevent the spread of the disease. The same epidemic, commented upon by Murata (1904) affords the figures 0·06 per cent. and 42·5 per cent. of morbidity and case mortality in the inoculated, to set against 0·13 per cent. and 75 per cent. in the uninoculated. These are not specially favourable figures except perhaps for mortality and are probably highly selected in one direction or the other. The caustic remark too of Sticker (1912), that for inoculation statistics it is the most favourable examples that are chosen, seems only too true. Probably the most satisfactory data relating to cholera inoculation are those given by Russell (1927) of an attack incidence of 0·41 and 0·49 per cent. among individuals inoculated with anticholera vaccine (parenteral) and bilivaccine (oral) respectively as contrasted with 2·1 per cent. among the non-inoculated.

As regards duration of immunity, the following are the types of estimate given : Haffkine and his co-workers, 14 months ; Kolle and Konrádi, each 12 months ; Barykin, 7 to 9 months. What seems certain is that the immunity is of short duration. It would follow, then, that for the individual or individuals exposed to continuous risk, repetition of

inoculation is essential after an interval depending on the season of prevalence of cholera and the degree of risk which is run. Konrádi (1915–16) advocates the use of a small yearly repeated dose. However much one may deplore the absence of the statistical evidence for the efficacy of cholera vaccine, it would seem wise for those who are in any degree constantly exposed to danger, or who are passing into a country in which the disease is common and endemic, or in whose neighbourhood cholera has appeared in epidemic form, to be inoculated with cholera vaccine, and, under the same circumstances, to have at least a small dose yearly for the maintenance of protection. The adoption of this procedure should not, however, be allowed to give a false sense of security, and all the precautions which the ætiology, pathology and epidemiology of cholera suggest should be adopted in the presence of an epidemic.

Vaccinotherapy, as might be expected in an acute disease like cholera, has found little or no place. One can only say that it has been tried (Babes, 1914) and has been claimed to have resulted in diuresis and improvement and to have helped other measures in the reduction of mortality.

Passive Immunity.

Lazarus (1892) showed that the blood-serum of convalescents from cholera possessed protective characters for the guinea-pig which had been injected with virulent cholera, and that this was not possessed by normal human-serum. The observation was made at a time when great things were expected of antisera. Since that time the view has more or less crystallized that, while passive immunity can be induced in animals and in man, it owes its greatest success, its only success apparently, in practice, to such antitoxic as distinguished from antibacterial characters which it may possess. The antibacterial characters of a serum are largely summed up in its content of agglutinins and bacteriolysins. These disappear rapidly on injection into a fresh animal. Their effect can be demonstrated in mixtures of 5 to 10 times the lethal dose of vibrios and serum, but they are of little avail in a disease like cholera to counteract the activities of the organism, when the infection precedes the administration of the serum.

Most of the therapeutic trials of cholera antisera have been made with antisera obtained in response to injection of animals with the toxin of specially toxic species of vibrio which are allied to or identical with the cholera vibrio. It is contended by Kraus and others that the sera obtained with vibrios such as El Tor, Nasik, &c., are capable of neutralizing the toxic action of the cholera vibrio on which the symptoms of the disease depend. Thus the whole question of the effectiveness of a cholera antiserum reverts to that of the nature of the toxin which acts in cholera, and indeed, it is the answer to this question which will settle the real nature of cholera immunity. It has been both maintained and denied that a cholera antiserum has antitoxic qualities. Pfeiffer (1894) distinguished toxic substances of a primary and secondary type and yet both derived

only from the bodies of the vibrios. The primary, almost insoluble, toxins are set free on the death of the injected bacteria in the animal body, and produce a powerful paralysing action on the centres of circulation and of temperature regulation. They are extremely labile. Besides these, there are the secondary toxic bodies which are by no means so toxic, and present the distinguishing characteristic of being relatively stable and capable of resistance even to the temperature of boiling water. In this observation, made with regard to the standard cholera vibrio, may perhaps be found the key to the differentiation of the cholera-like vibrios in general into those possessing highly toxic characters such as the El Tor vibrios, and perhaps referable to the production of specially large quantities of labile primary toxin, and those which are not toxic in the same sense, but still do possess toxin firmly bound to the protoplasm of the vibrios. The various strains may differ greatly in their capacity for production of labile toxin whether it be extracellular or intracellular. We have seen also that the standard cholera vibrio, although of double antigen type, possesses a comparatively small amount of thermo-labile antigen. The whole question, as we have said, of specifically antitoxic sera in cholera, as distinguished from anti-endotoxic or antibacterial, turns on the identity of cholera toxin. It is legitimate to doubt indeed whether the strict differences drawn between endo- and exotoxins themselves really do exist. All toxins may be disintegration products of bacteria rather than secretions. An antibacterial serum requires, according to the terminology of Ehrlich, conjunction with the non-specific complement of the serum to act bacteriolytically, and insufficiency of complement together with rapid excretion is held by some to account for the ineffectiveness of such cholera-serum (Baumgarten, 1921) to produce the bacteriolysis which should lead to disappearance and cure of the infection. The results of serum therapy in man have not been at all convincing. A passive immunity as a purely protective measure is ruled out of court because of its short duration.

The story of anticholera sera has been one of preparation of a serum from the vibrios themselves (antibacterial), from cholera endotoxin or from the toxin (antitoxic) of a toxic species of vibrio such as Massauah, Nasik or El Tor. The antibacterial serum of Pfeiffer could protect guinea-pigs against choleraic peritonitis, but not, as was shown by Metchnikoff, against intestinal infection which is regarded as a pure toxicosis. Metchnikoff, Roux and Taurelli-Salimbeni (1895) claimed to have obtained a toxin-producing cholera vibrio after its cultivation *in vivo* in collodion sacs. Horses intravenously immunized with this toxin furnished a serum which was bactericidal and agglutinating and was, therefore, antibacterial. It was also capable of protecting against intoxication produced by germ-free filtrates. Macfadyen (1906[1]) and Rowland obtained a powerful toxin (endotoxin) by their treatment of cholera vibrios at the temperature of liquid air and trituration. The serum obtained with this toxin was used by Macfadyen in human cases

(1906[2]). Brau and Denier (1906) then claimed to have obtained a soluble toxin, by which is meant a true toxin, by cultivating the cholera vibrio in a fluid medium of horse-serum, 90 parts, and defibrinated blood, 10 parts. This was also the claim of Kraus and Russ (1908). Most of these claims refer only to special strains of the cholera vibrio or even to vibrios which are rather to be regarded as allied to the cholera vibrios and specially toxic. Thus, Kraus definitely used the El Tor vibrio as the producer of the toxin with which he obtained his antitoxic cholera-serum. There is no need to multiply examples. They represent the endeavour especially to find an antiserum with definitely neutralizing character towards the supposed true toxin of the cholera vibrio. The sera which have been used therapeutically are mainly those of Kraus and Schurupoff.

The success of cholera-serum therapy has been altogether doubtful. At the outside it is claimed that the administration of the cholera-serum in large quantities has done no harm. But large quantities of serum, just as large quantities of salt solution, ought to do good in simple replacement of fluid which has been lost, and even as food. In the absence of sufficient knowledge of what the so-called cholera toxin is, the difficulty of proving antitoxic action in animals and the absence of any controlled statistics for the effective use of anticholera-serum in man, we may conclude that our knowledge on the subject of cholera therapeutic antiserum is very deficient.

Virulence of the Causal Organism.

The subject of virulence of the cholera organism, as the reverse of immunity or resistance of the individual to attack, may just be referred to in conclusion. There is considerable evidence to indicate that the cholera vibrio is an organism which is still in a state of transition from the extracorporeal non-pathogenic type of bacterium to the wholly parasitic type which may or may not be able to revert to the non-parasitic. We have evidence of cholera-like organisms apparently less virulent for man than the true cholera vibrio although at the same time more capable of producing toxic substances in culture media, of which the paracholera vibrios of Kraus, Castellani and Mackie and the ' half parasites ' of Bail may be instanced as examples. Their action on man is evidenced by the sporadic nature of the outbreaks in which they are met with and in the difficulty sometimes experienced of characterizing the disease as cholera. There is, further, the subject of the heightening of virulence by symbiosis and the virulence associated with the rough and smooth colony forms of the standard cholera vibrio, of which the smooth colony type would correspond to the common parasitic form and the rough colony type to the form which is more resistant to external influence but is at the same time correspondingly less virulent. These matters are likewise linked up with resistance and susceptibility to bacteriophage or lytic action and to lysogenic character. We have also the contention put forward that the inagglutinable, avirulent vibrio of the village water tanks of epidemic

times is the true cholera vibrio in its non-parasitic form—perhaps an avirulent resistant vibrio developed, as R forms may be, by growth in immune fluids in the body of the individual who has recovered and become immune for the time being to cholera. Lastly, it is maintained (d'Herelle and Malone, 1927) that localized epidemics may come to an end with the passage into water supplies or other contaminating sources of bacteriophage from convalescent cases along with the infecting cholera vibrios.

Paracholera and the 'Paracholera Vibrios'.

By T. J. Mackie.

The term paracholera was first applied by Kraus (1909) to infections by vibrios which, though closely related to *V. choleræ*, differed in their hæmotoxic action, viz. strains of the El Tor type. The El Tor vibrio, however, in view of its serological reactions is now generally regarded as specifically identical with *V. choleræ*. Reference has been made in previous sections to the original discovery of this organism, its occurrence, biological characters, toxigenic properties and serological reactions. The essential difference of this organism from the typical cholera vibrio lies in its pronounced and rapid hæmolytic action and the formation of a diffusible toxin which is rapidly lethal to laboratory animals on intravenous injection. It was originally found in dysenteric cases showing no signs of cholera, though the particular patients may have been cholera carriers. It may thus represent a form of *V. choleræ* modified by continued residence in the bowel.

As regards pathogenicity to the human subject, the El Tor vibrio does not occur as a prevalent organism in typical epidemic cholera, and has never been isolated from cholera cases in India (Greig). Vibrios corresponding to the El Tor organism have been met with, however, in sporadic choleraic conditions and in carriers in other countries, e.g. the Near East. A similar type designated 'kadikoj' was isolated by Kraus in 1913, in Bulgaria (see Kovács, 1926). The El Tor organism might be regarded as a variant of *V. choleræ*, of reduced virulence for the human subject.

Reference has also been made (p. 341) to 'paracholera vibrios' which are specifically different from *V. choleræ*, and the term paracholera has been applied by some writers (see Mackie, 1922) to infections regarded as bearing the same clinical and bacteriological relationship to true cholera as paratyphoid infections have to typhoid fever. In this sense the name '*V. paracholeræ*' is applied to vibrios which are serologically distinct from *V. choleræ*, and its use conforms to previous custom in bacteriological nomenclature. This application of the name paracholera seems, therefore, more justifiable than that adopted by Kraus.

Castellani, in 1916, applied the term paracholera to cases hardly distinguishable from true cholera in their clinical manifestations but due to vibrios which were biologically different from *V. choleræ*. He suggested

that choleraic disease is produced by a group of vibrios, not by one specific type. The organisms which he referred to as paracholera vibrios were : Finkler and Prior's vibrio (*V. proteus*) originally isolated from a case of ' cholera nostras ' in Germany (p. 432) ; *V. massauah*, isolated in 1895 by Pasquale at Massauah, from a case of ' clinical cholera ' (p. 431) ; the El Tor vibrios as described by Gotschlich (p. 364) ; and a vibrio which he designated *V. kegallensis*, found in Ceylon in cases clinically indistinguishable from true cholera, with profuse diarrhœa, severe vomiting, muscular cramps and other choleraic symptoms. In such cases this organism was present in large numbers in the stools and was the only species of vibrio present. He also recovered it from well water which seemed to have some epidemiological relationship to the condition. It resembled *V. choleræ* in general characters but produced no indole or only a slight trace, and it was entirely different in serological characters ; hæmolysis was inconstant ; milk was acidified and coagulated. Thus Castellani has included among the paracholera vibrios both the El Tor organism which is serologically identical with *V. choleræ* and also serologically distinct organisms. It would be more appropriate as suggested above to apply the name only to organisms that can be clearly differentiated from *V. choleræ*.

Chalmers and Waterfield (1916) described a vibrio strain causally associated with a case of paracholera at Port Sudan in 1915, and claimed to have identified it with *V. gindha* (Pfeiffer), which was originally found in the water of a well at Gindha (in Erythrea) after an epidemic of ' cholera '. Their case was more of the nature of a severe diarrhœa than true cholera, but the patient suffered from intense colic, muscular cramp and vomiting and became collapsed like a case of typical cholera. It is of interest to note that a severe choleraic condition in the human subject followed the ingestion of a culture of the original *V. gindha* (see Macé, 1913). Chalmers and Waterfield's strain resembled the cholera vibrio, and gave the cholera-red reaction, though irregularly, but was serologically distinct from *V. choleræ*. It produced rapid liquefaction of coagulated serum and was actively hæmolytic on blood-agar plates like the El Tor vibrio. Glucose, lactose, saccharose and mannitol were fermented (without gas production) and milk was acidified and coagulated. On alkaline potato a maize-yellow growth resulted. In their paper, Chalmers and Waterfield carefully reviewed previous literature bearing on the occurrence of non-cholera vibrios in choleraic cases, healthy persons, water, &c., and discussed the biological classification of the various types of vibrios that had been described before this date.

Two serological races of paracholera vibrios associated with a limited choleraic outbreak in Egypt in 1916, were described by Mackie and Storer (1918), and for purposes of reference were designated ' *V. paracholeræ* A and B '. These corresponded closely in general characters (including biochemical reactions) with the cholera vibrio, but differed in being actively hæmolytic both on blood-agar and when tested by Greig's method (p. 366). They were not agglutinated even in low titres by antisera to known

V. choleræ strains, and antisera for both types respectively failed to react with *V. choleræ*. These strains were all highly virulent to guinea-pigs by intraperitoneal injection, and to rabbits by intravenous inoculation, though non-pathogenic to pigeons by intramuscular injection of small doses. They occurred in large numbers in the dejecta of the cases, and in each case the particular vibrio was the only species present. The serum of one case agglutinated the associated organism in a dilution of 1 : 200 after 7 days from the onset of the illness. [Konschegg and Weltmann, 1913, also described a non-cholera vibrio strain '*V. favorit*', which was agglutinated by a 1 : 400 dilution of the serum of the person from whom it was isolated.] In a further study of paracholera cases in Egypt (1916–18), 57 vibrio strains in all were carefully investigated by Mackie (1922) ; these were biologically similar to the types originally described. They were isolated from typical choleraic cases, non-choleraic diarrhœa and from healthy carriers who were contacts of choleraic cases, ' cholera ' convalescents and cases of ' acute diarrhœa '. These strains were classified serologically, and among them 20 different types were identified. Certain types were represented by single strains only. The '*V. gindha*' of Chalmers and Waterfield was serologically identical with one of these types. The serological differentiation of these organisms from *V. choleræ* was well marked. It was concluded from the evidence available that vibrios with the group characters referred to were responsible for a condition varying from a simple diarrhœa to an acute illness clinically indistinguishable from true Asiatic cholera. The cases, however, were sporadic or occurred in limited outbreaks. Fatal results, though uncommon, were recorded among the series of cases studied.

Greig in 1917, after a long and extensive study of the ' cholera-like vibrios found in the stools of cholera cases in India, concluded that in addition to typical cholera produced by *V. choleræ*, cholera-like infections of milder type also occur associated with the presence of monoflagellate vibrios closely resembling the typical cholera organism, but clearly differentiated from it in serological characters. These organisms were found in cases usually during the decline of a cholera epidemic and rarely at the early stage of an epidemic when the mortality was high. Greig (1914[3]) also recorded the occurrence of such vibrios as concomitants of the true *V. choleræ*. Seventy-five strains of ' cholera-like ' vibrios were studied serologically, and 62 of these could be classified into 9 groups, of which one was most prevalent in Calcutta.

There can be little doubt, therefore, of the existence of a choleraic condition due to vibrios which are serologically different from *V. choleræ*, and the recognition of ' paracholera ' seems justified. Japanese workers have described, however, three serological types of '*V. choleræ*', and the question arises as to the relationship of these types to the typical cholera vibrio of India and the paracholera vibrios. It is of interest that the paracholera organisms generally possess the rapidly active hæmolytic properties originally described in the case of the El Tor vibrio.

Similar strains have frequently been isolated from water and were originally studied by Sanarelli (1893), Wernicke (1894), Kutscher (1895) and Dunbar (1896). The question of the biological relationships of such organisms and of the extracorporeal transformation of *V. choleræ* by residence in water has been discussed in a previous section. Vibrios derived from water supplies in India have been carefully studied by Greig (1916) ; they resemble *V. choleræ* in biological characters and in optimum temperature for growth but differ in agglutinability by an anticholera-serum, and possess a specifically different agglutinogen. They are also as a rule actively hæmolytic and correspond, therefore, to the paracholera vibrios described above.

While some vibrios isolated from water are purely saprophytic, growing only at low temperatures (vide p. 435) many such organisms may only be temporarily water organisms and may be of intestinal origin. It might be supposed that there exists a large group of closely related vibrios varying in parasitic properties and in potential pathogenicity. Of these *V. choleræ* is the most highly specialized in parasitism and pathogenicity ; others may possess a somewhat lower aggressiveness and pathogenicity, e.g. the paracholera vibrios ; some species are purely saprophytic, while, others again may occupy an intermediate position between the truly parasitic forms and such saprophytes, and it is possible that these organisms may establish themselves in the intestinal tract with or without pathogenic effects.

The question of the occurrence of commensal vibrios in the intestine was investigated in Egypt in 1916 by Mackie during a time when paracholera was occurring among British troops, but among a large series of native Egyptians in normal health no vibrios were found in the stools.

The diversity of serological types among these organisms, and the occurrence of serologically individualistic strains is, of course, paralleled in many other bacterial groups.

During the cholera epidemic of 1902 in Egypt, many ' cholera-like ' vibrios were encountered by Kolle and Gotschlich (1903) in the course of their bacteriological investigations, and were classified as follows (see also Chalmers and Waterfield, 1916) :

A. Polytrichous (' *Polytricha* ').

Long thin rod-shaped vibrios with 2 to 8 terminal flagella.

1. Cholera-red reaction positive ; markedly virulent for animals by experimental inoculation.

2. Cholera-red reaction negative ; virulence weak.

B. Monotrichous (' *Monotricha* ') (*with one polar flagellum*).

1. Virulent for pigeons, i.e. corresponding to *V. metchnikovi* (vide *infra*).

2. Non-virulent for pigeons. In this group morphology varied—some strains showing long thin forms, others short rods ; the cholera-red reaction also varied.

All these organisms were differentiated from *V. choleræ* by serological reactions; they were isolated both from the dejecta of choleraic cases and from healthy persons. They were not apparently regarded as pathogenic, and it was suggested that they were saprophytic forms that had gained access to the alimentary tract. This question has been referred to above. Certain of these organisms, however, correspond to the paracholera vibrios described by later observers, and in view of later work it seems possible that such 'cholera-like' vibrios included types capable of setting up a choleraic condition.

Among the comma-shaped, monoflagellate, non-phosphorescent vibrios which were non-virulent to pigeons, liquefied gelatin, produced indole and failed to coagulate milk, a subgroup ('Group IV') was recognized by Ruffer (1907[1 & 2]) which also corresponds to the paracholera vibrios, resembling *V. choleræ* in biological characters except for their hæmolytic action and inagglutinability by an anticholera serum.

An organism which was isolated from a choleraic case and designated '*Vibrio nasik*' from its place of origin in India, corresponds closely to the paracholera vibrios described above. It was specially studied by Kraus (1920) as regards its toxigenic properties, in which it resembles the El Tor organism. In bouillon cultures after 3 to 4 day's growth, an active toxin could be demonstrated, which injected intravenously in small doses into rabbits produced a lethal effect in 10 to 15 minutes. The toxin was also active when introduced subcutaneously. According to Kraus it was inactivated at 58° C., and by various chemicals, e.g. chloroform. Filtrates of cultures were strongly and rapidly hæmolytic. Kraus obtained a specific antitoxin by immunizing animals. The serological reactions of this organism have been studied recently by Douglas (1921) who found it had no relationship to *V. choleræ*.

It seems possible, therefore, to recognize tentatively a 'paracholera group' differing from *V. choleræ* mainly as regards hæmolytic properties and serological characters, the hæmolytic effects of these organisms resembling that of the El Tor vibrio. This paracholera group would, therefore, include the 'cholera-like' organisms described by Greig, the '*V. gindha*' of Chalmers and Waterfield, *V. kegallensis* (vide *supra*), the paracholera vibrios described by Mackie, certain of the 'cholera-like' vibrios of Kolle and his co-workers, and Ruffer's Group IV. There is evidence, however, that organisms of the following types may be associated with diarrhœal and choleraic conditions: *V. proteus*, *V. metchnikovi*, *V. massauah*, *V. septicus*, *V. phosphorescens* (vide *infra*), and this would, of course, entail a broader characterization of the paracholera group. The whole subject of paracholera requires further study, and there is great need for correlated observations in different countries regarding the prevalent types of vibrios isolated from typical cholera and the less severe cholera-like conditions.

Representative Types of the Group of Vibrios.

By T. J. Mackie.

The group of vibrios constitutes one in which biological classification presents many difficulties. It has been shown how *V. choleræ* exhibits considerable variability in its biological characters, and it is difficult, therefore, to assess the importance which can be attached to certain characters on which stress has been laid in regard to differentiation of species. Further, many of the types recorded in earlier literature were only studied with reference to a limited set of characters and the records of these lack details which would now be considered necessary for any exact comparison with more recently described strains. Some varieties, in fact, are only recorded in the earliest bacteriological literature and strains are not now available for study. Their identity and relationships, therefore, remain doubtful. A profuse nomenclature has also developed progressively in the literature of the subject, numerous strains having been isolated which were regarded by workers, often erroneously, as new types and given, therefore, specific names. The various recorded species have recently been described in detail by Ford (1927). Vibrios, as indicated in an earlier section, have been isolated from widely different sources and in discussing the group as a whole, particularly in relation to the cholera and the paracholera vibrios, certain representative types, which have been specially studied and recorded in the literature under particular names, merit some consideration. Only undoubted vibrionic types will be discussed and forms which can be definitely classified as spirilla are not dealt with here. It must be recognized, however, that there is no sharp line of demarcation between the two genera. These organisms are grouped for convenience of description as follows :

1. Those of high virulence for guinea-pigs and pigeons, such as *V. metchnikovi ;* the grade of virulence which these organisms possess for the particular animals is of a remarkably high order (vide *infra*) and seems to justify their being grouped together.

2. *Vibrio gindha* and allied types, which resemble *V. choleræ* in general characters but differ from it serologically ; this group would include certain of the paracholera vibrios which have been described above.

3. *Vibrio proteus* and vibrios which fail to yield the nitroso-indole reaction ; most of these organisms are non-pathogenic to laboratory animals.

4. Phosphorescent vibrios.

5. Vibrios described as possessing special chromogenic properties.

6. Non-proteolytic vibrios (including also non-saccharolytic types).

7. Water vibrios growing only at low temperatures.

8. ' *Vibrio nasalis* '—a large non-motile vibrio described by Weibel in 1888.

9. Vibrios isolated from the excreta of swine and from manure.

10. Vibrios isolated from fish and molluscs.
11. *Vibrio fœtus* of infectious abortion of cattle.
12. Anaerobic vibrios.

Vibrio metchnikovi and Related Vibrios (Vibrios of High Virulence for Pigeons or Guinea-pigs).

Vibrio metchnikovi was first described by Gamaléia in 1887, at Odessa, as the cause of epizootic enteritis of fowls, a condition not unlike 'fowl cholera'. The organism occurred in the intestine and in the blood. It has a single terminal flagellum and is morphologically similar to *V. choleræ*. In other biological characters it also resembles the cholera vibrio and it gives the nitroso-indole reaction. It is, however, serologically distinct from *V. choleræ*. The results of experimental inoculation have elicited a striking difference between this type of vibrio and *V. choleræ*, as originally shown by Pfeiffer and Nocht (1889). An exceedingly minute quantity of culture introduced into a skin wound in pigeons leads to a fatal septicæmia, while, by the same method, *V. choleræ* is quite ineffective even in large doses. It was stated, however, by Gamaléia and by Salus (1893) and Weibel (1894) that the virulence of *V. choleræ* could be so exalted by passage in pigeons as to exhibit the same degree of pathogenicity as *V. metchnikovi*. In guinea-pigs, *V. metchnikovi* is also more virulent than the cholera vibrio; thus, a septicæmia follows subcutaneous injection. In guinea-pigs an acute gastro-enteritis was produced by oral introduction of culture after alkalinization of the stomach secretion, and the vibrio occurred in large numbers in the intestinal contents, in the blood and internal organs. Guinea-pigs have also been infected *per os* without neutralization of stomach contents. Pigeons could not be infected by oral administration even when large doses were given. Fowls were found to be less susceptible than pigeons on subcutaneous and intramuscular injection, but cultures were pathogenic when introduced *per os*.

A standard strain of this organism (from the National Collection) shows the same sugar reactions as *V. choleræ* and is strongly hæmolytic towards ox- and rabbit-blood when tested by Greig's method.

This type of vibrio has also been isolated from the human subject. Thus, Gieszczykiewiez and Sierakowski (1915) described an organism of the *V. metchnikovi* type in a diarrhœal condition with 'rice-water' stools. This organism was serologically distinct from a standard *V. metchnikovi* and *V. choleræ*, but possessed a high virulence for guinea-pigs and birds. Horst (1922) isolated a similar organism from a liver abscess ('*V. leidensis*').

A vibrio named '*V. schuylkilliensis*' was isolated by Abbott, from the Schuylkill River, at Philadelphia, in 1896, and a similar organism was described by Abbott and Bergey (1897) as occurring in the river water at various times of the year and in the sewage entering the river. This organism resembled *V. choleræ* in general biological and biochemical characters, but was differentiated clearly by serological reactions. In

pathogenesis to animals it corresponded to *V. metchnikovi*, and in cross-immunity experiments it seemed to be identical with this organism.

Vibrio massauah was found by Pasquale (1891) in the stool of a choleraic case at Massauah, and resembled *V. choleræ* in many respects but differed in possessing multiple flagella (numbering up to 4). Liquefaction of gelatin was slow and incomplete. The cholera-red reaction was positive. This organism was differentiated from *V. choleræ* by immunity reactions. In animal inoculation tests it behaved like *V. metchnikovi*, being highly virulent to pigeons and guinea-pigs by intramuscular and subcutaneous injection. A choleraic condition in young rabbits was produced by feeding with culture.

V. danubicus was isolated by Heider (1893) from the Danube canal at Vienna. It resembled *V. choleræ* in most of its general characters, but was virulent to pigeons (though not so markedly as *V. metchnikovi*) and also to guinea-pigs by intraperitoneal injection, by oral administration after alkalinization of the stomach contents, and by injection directly into the duodenum. It appeared to be related to *V. metchnikovi*.

Vibrio septicus is of special interest in view of its remarkably high virulence for guinea-pigs. It was found by Kolle in a case of ' cholerine ' in Danzig, and corresponded in morphological and cultural characters to *V. choleræ*, but was not agglutinated by anticholera-serum. For pigeons it was not virulent, but it killed guinea-pigs in most minute doses when introduced into a wound of the skin, death resulting from septicæmia in 4 to 6 hours (see Kolle and Prigge, 1927).

Vibrio gindha and Allied Types.

Vibrio gindha was isolated by Pasquale (1891) from the water of a well at Gindha, near Massauah. This water supply was suspected of having originated a choleraic outbreak. The organism was carefully studied by Pfeiffer (1896) and resembled *V. choleræ*, but differed in immunity reactions. It was found to be toxic to guinea-pigs on intraperitoneal injection, but was not virulent for pigeons and guinea-pigs when injected subcutaneously or intramuscularly. It possessed a single terminal flagellum. The nitroso-indole reaction was variable.

A vibrio isolated from water by Kutscher was named by Migula (1900) *Microspira wieseckensis*. This organism has been grouped by Chalmers and Waterfield along with *V. gindha*. It was described as liquefying gelatin like *V. choleræ*. It gave the nitroso-indole reaction and liquefied serum (slowly).

Vibrio berolinensis was isolated by Neisser (1893) from filtered Stralauer water to which *V. choleræ* had been added for experimental purposes. It appeared similar to the typical cholera organism in all its biological characters but proved practically non-pathogenic to laboratory animals.

Chalmers and Waterfield (1915) identified as *V. gindha* a paracholera vibrio isolated at Port Sudan. This strain has been found to be serologically identical with certain paracholera vibrios isolated in Egypt by Mackie (p. 425).

Many of the paracholera vibrios would in fact fall into line as regards biological characters with the original *V. gindha*.

Vibrio proteus and Allied Types.

Finkler and Prior's vibrio (*V. proteus*) was originally isolated from the dejecta of a case of 'cholera nostras' in Germany by Finkler and Prior (1884). It was found to be similar to *V. choleræ* but did not exhibit the cholera-red reaction, indole and nitrite formation being absent. It was described as producing a brownish growth on agar and on potato. A vibrio strain designated '*Vibrio Finkler-Prior*' from the National Collection of Type Cultures has been found to possess multiple polar flagella. Emphasis has been laid on the behaviour of Finkler and Prior's vibrio in gelatin-stab culture and it has been definitely contrasted with *V. choleræ* in respect of its rapid saccate liquefaction (p. 352). The question arises, however, whether differences in the type of liquefaction of gelatin can be regarded as specific features. It was found that this organism on intraperitoneal injection in guinea-pigs was virulent but apparently to a lesser degree than *V. choleræ*. The National Collection culture ferments glucose, mannitol and maltose (without gas production) and has no action on lactose, dulcitol and saccharose (cp. *V. choleræ*). It is not hæmolytic on blood-agar or when tested by Greig's method ; this applies to the blood of various animal species.

Vibrio tyrogenus was isolated by Deneke (1885) from cheese and originally attracted special attention in view of its biological relationship to the cholera vibrio. It is now mainly of historical interest. A culture in the American Museum has recently been described by Ford (1927) : in morphology it corresponds to *V. choleræ* but it does not liquefy gelatin or coagulated serum ; the nitroso-indole reaction is negative, and glucose, lactose and saccharose are not fermented. It was originally described, however, as liquefying gelatin. Slight pathogenicity to guinea-pigs on intraperitoneal injection was recorded and it was found that if the gastric contents were neutralized and the intestines paralysed by opium, 20 per cent. of the animals died, as in Koch's classical experiments with *V. choleræ*.

Vibrio aquatilis, isolated by Günther (1892) from the river Spree, near Berlin, and also found by Kiessling (1892–3) at Altona, was morphologically like *V. choleræ* with one terminal flagellum though described as tending to form thick spirals reaching 80μ in length and aggregated in tangled masses. It grew well at 37° C. Gelatin was liquefied. The nitroso-indole reaction was negative with bouillon cultures, though the formation of indole and nitrite was demonstrated in agar cultures. The organism was non-pathogenic to laboratory animals.

Vibrio sputigenus. This organism was isolated by Brix (1894) from the sputum of a case of pneumonia. It resembled *V. choleræ* in biological characters but produced more rapid liquefaction of gelatin and failed to give the nitroso-indole reaction. It was non-pathogenic.

Vibrio milleri was described (Miller, 1885) as occurring in carious teeth. It did not give the nitroso-indole reaction and was non-pathogenic. A culture in the American Museum has been studied by Ford (1927). In morphology it shows no essential difference from *V. choleræ*, but the colonies on gelatin differ from those of other vibrios in their feathery appearance. Gelatin is liquefied rapidly. On agar and potato a yellowish growth results.

Vibrio wolfii was isolated by Wolf (1893) from the uterine exudate of a case of endometritis. In morphology and cultural characters it was similar to *V. choleræ* but it coagulated milk and failed to produce the nitroso-indole reaction. It proved pathogenic to mice by inoculation of large doses.

Vibrio striatus was isolated from water by Kutscher and named *Microspira striata* by Migula (1900). It liquefied gelatin like *V. choleræ* but did not yield the cholera-red reaction and produced no evident growth on potato.

Phosphorescent Vibrios.

Vibrio phosphorescens was first isolated by Dunbar and Rumpel during the summer of 1893, from the waters of the Elbe, Havel, Rhine and Spree, and was studied by Rumpel (1895), Dunbar (1896) and others. It was characterized by the phosphorescence of bouillon and gelatin cultures at 22° C. Though not agglutinable by anticholera-serum it was found to be pathogenic (like *V. choleræ*) to laboratory animals, and apart from phosphorescence resembled the true cholera vibrio in general biological characters. Similar vibrios have been reported by various other writers as occurring in water and in the fæces from cases of diarrhœa. This type of organism is classified as '*V. albensis*' by Lehmann and Neumann (1927).

The writer isolated strains of phosphorescent vibrios from the stools of persons suffering from acute choleraic diarrhœa in Egypt (1917). Apart from their phosphorescent character they resembled the paracholera vibrios described above.

Jermoljewa (1926) has recently recorded the occurrence of *V. phosphorescens* in cases with symptoms of cholera. He has also noticed the agglutination of these organisms by the serum of the cases from which they were isolated. In one patient the vibrio was isolated from the bile *post mortem*.

Vibrios described as Possessing Special Chromogenic Properties.

While the cholera vibrio and related organisms possess some degree of chromogenic power, more evident in growth on alkaline potato than on ordinary media, certain types of vibrios have been described with marked pigmentation of growths on ordinary nutrient agar and even in alkaline peptone water. The formation of pigment in alkaline peptone water has been emphasized by Chalmers and Waterfield as a special character of a

group of vibrio which they proposed should be classified under the designation of '*V. drennani*'. The type strain is a vibrio described by Drennan (1914) isolated from the rectal contents of a person examined at the Quarantine Station, New York, suffering from some 'intestinal disturbance'. It proved to be a motile, monoflagellate, Gram-negative vibrio producing on agar a large white moist colony turning slowly to a dark-brown colour. In alkaline peptone water the same dark-brown coloration occurred both in the surface pellicle and in the depths of the tube. It liquefied gelatin slowly, produced acid in glucose and saccharose but not in lactose, and was slowly hæmolytic. No indole was formed. Formation of pigment in peptone water would undoubtedly constitute a striking difference from *V. choleræ* and allied organisms.

Craster (1913) also described non-cholera vibrios with marked pigment formation.

Chromogenic vibrios were described by Weibel. '*Vibrio aureus*' (Weibel, 1888) was isolated from canal-mud and grew at 22 and 37° C. It was stated to be a non-motile, Gram-negative vibrio producing on culture medium a golden-yellow pigment. '*Vibrio flavus*' of similar origin (Weibel, 1888) differed from '*V. aureus*' in the ochre-yellow colour of its pigment. Fuhrmann (1905) has recorded a water vibrio ('*V. aquatilis fluorescens*') characterized by the formation of a green fluorescent pigment.

Vibrios devoid of Proteolytic Properties (including also Non-saccharolytic Vibrios).

Vibrio iners was reported by Besson, Ranque and Senez (1918) as frequently present in summer diarrhœa. It is described as a motile, Gram-negative vibrio occurring in short or long forms and with 1 to 4 terminal flagella; it does not liquefy gelatin and has no proteolytic or saccharolytic properties. The growth in culture media resembles that of the typhoid bacillus. It is non-virulent to guinea-pigs on intraperitoneal injection. This type of organism differs in many important features from other vibrios.

Bacillus fæcalis alkaligenes has been regarded by some workers as more allied to the vibrios than to the coli-typhoid group with which it is often grouped. Many strains designated *B. fæcalis alkaligenes* possess terminal flagella (1 to 6) like vibrios (Berghaus, 1905; Klimenko, 1907; see also Lehmann and Neumann, 1927) and, though more frequently appearing as straight rods, may show typical vibrionic or spirillary individuals. *B. fæcalis alkaligenes* is frequently found in large numbers in the dejecta in diarrhœal conditions and it seems not improbable that '*V. iners*' is closely related to this organism, in view of its complete lack of fermentative properties. An organism named *Vibrio tonsillaris* (Stephens and Smith, 1896) found in the throat along with the diphtheria bacillus was probably also of the same type. Another similar organism is that described under the name of '*V. terrigenus*' by Gunther in 1894. It did not liquefy gelatin and the nitroso-indole reaction was negative.

Vibrio bonhoffii was isolated by Bonhoff (1893) from the water at Stolp in Pommerania. This strain resembled *V. choleræ* in many of its reported biological characters but apparently failed to liquefy gelatin or to coagulate serum.

Vibrio surati was obtained by Lamb and Paton (1913) from a vegetative endocarditis and was present also in the blood. In morphology it showed typical comma-shaped forms and also long spirilla. It was motile and Gram-negative. It grew feebly on plain agar but on ascitic-fluid agar produced small semi-transparent dew-drop-like colonies. No liquefaction of gelatin occurred and no indole formation.

Vibrio percolans was isolated by Mudd and Warren (1923) from hay infusion and is of special interest in view of its ability to pass through a Berkefeld filter. It is a typical Gram-negative vibrio 0·5 to 2·5μ in length by 0·3μ broad and has 1 or 2 terminal flagella. The optimum temperature is 30° C. It does not grow well in ordinary media but better in a neutral hay infusion. Gelatin is not liquefied. Indole is not formed and no fermentation of sugars has been noted. It is non-pathogenic. It is stated to be able to pass a Berkefeld V filter, though arrested by an N filter. It is significant that its passage through a filter was stopped by suppressing motility at low temperatures.

Water Vibrios growing only at Low Temperatures.

Vibrio marinus, isolated by Russell (1891) from sea-water at Naples, may be taken as a type of purely saprophytic water vibrio (see Ford, 1927). In morphology it appeared as a comma-shaped form and, when the individuals were attached end to end, as a typical spirillum. It grew only at low temperatures and not at 37° C. Gelatin was liquefied. Growth occurred best on media made with sea-water. On agar an abundant white growth resulted, on potato a reddish-brown growth with a greyish-green coloration of the medium.

Vibrio portuensis represents another type of saprophytic water vibrio. It was isolated by Jorge (1896) from a water supply in Portugal (see Ford, 1927). On first isolation no growth occurred above 30° C. It presented typical comma-shaped forms after repeated subculture, but also exhibited considerable pleomorphism and great tendency to involution. It possessed multiple flagella. Gelatin was not liquefied but the nitroso-indole reaction was noted. On potato the growth was of a brownish colour. Glucose and lactose were fermented. It was not virulent to guinea-pigs on intra-peritoneal injection.

Vibrio nasalis.

Vibrio nasalis was first described by Weibel (1887) in the nasal secretion. It was described as a large, thick, non-motile, Gram-negative vibrio which varied in its curvature. In thickness it was comparable to the anthrax bacillus and thus in morphology differed entirely from *V. choleræ*. In mucus and in culture, polar staining was noted. In cultures

on solid media long chains of curved rods and wavy or spiral filaments were found. Pleomorphism and involution were marked features in culture. A gelatin-stab culture resembled that of a streptococcus and no liquefaction occurred. Surface colonies were small brownish-yellow discs with sharply defined edges. No pathogenicity was demonstrated.

Vibrios Isolated from the Excreta of Swine and from Manure.

A number of vibrio types have been found in the intestine of swine and in manure. These have been described and named by Migula (1900). They have also been referred to by Ford (1927). Some of them closely resemble *V. choleræ* in morphology and cultural characters. Others were described as possessing multiple terminal flagella and exhibiting differences as regards optimum temperature for growth, absence of liquefaction of gelatin, absence of fermenting properties, non-pathogenicity to laboratory animals, &c. One type named *Microspira intermedia* by Migula and originally described by Kutscher (1895) closely resembled *V. choleræ* in general biological characters but grew better at low temperatures (e.g. 22° C.) than at 37° C. It was found, however, to be pathogenic to guinea-pigs, like *V. choleræ*, on intraperitoneal injection. Certain of these organisms correspond to the purely saprophytic types found in water and some to vibrios that have been isolated from human fæces (vide *supra*).

Vibrios Isolated from Fish and Molluscs.

Bergman (1909) described a vibrio which he isolated from an infective tumour of eels (*V. anguillarum*). He later reported the occurrence of a similar organism in an infectious disease of codlings (1912) in which destruction of the eyes occurred (keratomalacia). This latter organism was found to be a small, motile, Gram-negative vibrio which grew best at room temperature. Gelatin was liquefied, milk was acidified and coagulated, and various sugars fermented with gas production (glucose, lactose, saccharose and maltose). The nitroso-indole reaction was negative. Experimental inoculation of cultures into the cornea of various fish produced ocular changes similar to those noted in the disease from which the organism had been isolated. This organism was also pathogenic by subcutaneous inoculation. Bergman postulated the existence of a group of vibrios pathogenic for fish.

David (1927) has recently described a vibrio (*V. piscium*) in a disease of carp. This organism closely resembles *V. choleræ* but does not grow at 37° C. It produces hæmolysis on blood-agar after 3 to 4 days.

Vibrio cardii was isolated by Klein (1905) from the heart mussel (*Cardium edule*). It resembled *V. choleræ* in general morphology, was actively motile (with 1 to 2 terminal flagella) and liquefied gelatin like the cholera vibrio. Milk underwent coagulation. The nitroso-indole reaction was negative. This organism was pathogenic to guinea-pigs on intraperitoneal inoculation.

A somewhat similar vibrio was also found by Klein in *Mytilus edulis*. This organism, however, did not liquefy gelatin and was non-pathogenic.

Remlinger and Nouri (1908) have isolated vibrios from oysters.

Vibrio fœtus of Infectious Abortion of Cattle.

Vibrio fœtus was isolated by Smith in 1918 from cattle with a characteristic form of infectious abortion. It was studied in detail by Smith and Taylor (1919) and fully described. It has been isolated from the placenta and from the fœtus. *V. fœtus* is only dealt with briefly in this chapter as a representative of the biological group of vibrios and for comparison with other vibrios.

V. fœtus varies considerably in size. The average length in the fœtal fluids is 4 to 5μ; short forms, 1·5 to 2μ, may also be observed; the breadth is 0·2 to 0·3μ. Elongated spirals may be noted. The short forms are comma-shaped, the longer organisms exhibit 2 to 4 coils. *V. fœtus* is actively motile, especially the short forms, and shows a flagellum at one or both poles. It is Gram-negative. Deeply staining granules have been observed in the organisms when examined in old cultures. These probably represent some form of degeneration.

This organism was first cultivated in sealed agar slope tubes (containing condensation fluid) with a piece of fœtal tissue added to the medium. Development was not abundant and appeared after 3 or 4 days as greyish lines of growth spreading upwards from the condensation fluid at the edge of the slope. Growth extended between the agar and the glass but there was little tendency to spread on the surface of the medium. The organism is apparently micro-aerophilic. Growths have also been obtained in the condensation fluid of agar slopes by adding a few drops of sterile defibrinated horse-blood to the fluid. Repeated subculture increased the abundance of the growth under these conditions, and surface cultures were ultimately obtained (after prolonged cultivation) as greyish white films or separate round colonies, 1 to 2 mm. in diameter. After prolonged subculture growth was obtained on plain agar in sealed tubes. Saprophytized strains grew in bouillon to which blood was added, producing turbidity and a mucoid sediment.

No definite biochemical reactions, e.g. fermentation of sugars, &c., have been demonstrated. Different strains have been found to be identical in agglutination reactions. No effects have been produced experimentally in laboratory animals.

Anaerobic Vibrios.

Certain anaerobic vibrios have been described. These have been isolated mostly from the mouth and upper respiratory passages of the human subject, occurring as commensals or as secondary infecting organisms in pathological lesions. They are probably related closely to certain bacteria which have been generally classified as spirilla, e.g. *Spirillum sputigenum* (Miller, 1906). An anaerobic vibrio cultivated by Mühlens (1909) from

the mouth exemplifies this group. It was grown in serum agar and described as a delicate comma-shaped organism smaller than the cholera vibrio. It possessed terminal flagella.

Veillon and Repaci (1912) isolated an anaerobic type of vibrio (*V. tenuis*) from tuberculosis lesions of the lung. This organism appeared as very actively motile Gram-negative comma- and S-shaped forms with single polar flagella. It was also found in the mouth, carious teeth and dental abscesses. Growth resulted in deep tubes of peptone agar with 1·5 per cent. glucose and 1·0 per cent. potassium nitrate. Tunnicliff (1914) also described a strictly anaerobic vibrio isolated from the sputum of a case of bronchitis and probably closely related to Veillon and Repaci's organism. It was Gram-negative and usually 2 to 4μ long by 0·25 broad, with 1 or 2 curves. Chains and long filaments were also observed.

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CHAPTER VI. *PASTEURELLA* TREVISAN.

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Under this heading those members of the genus *Pasteurella* will be considered which form a group apart from *B. pestis* and *B. pseudo-tuberculosis rodentium*.

History.

The pasteurelloses caused by bacteria of this group are almost without exception diseases of the animal world. Hueppe (1886), working with the organisms of fowl cholera, rabbit septicæmia, hæmorrhagic septicæmia of cattle and swine plague which had been isolated by various investigators during the previous decade, found that these bacteria agreed in certain cultural characters and that the diseases caused by them closely resembled one another. He, therefore, regarded the four organisms as varieties of the same species, and bearing in mind the type of disease which they occasioned, gave them the name *B. septicæmiæ hæmorrhagicæ*. The bacterial characters on which he based his diagnosis were few; the organism was a short, bipolar-staining rod, non-motile and with a tendency to produce involution forms. By stressing the nature of the pathological processes in the animal and relying on the simple bacteriological characterizations, Gram-negativity, ovoid form and bipolar staining, investigators were led to include under the same species-name strains which were biologically very wide apart.

After examining the organisms isolated from a variety of animal diseases of a hæmorrhagic nature, Lignières (1900) defined their biology more closely and adopting the nomenclature proposed by Trevisan gave them in commemoration of Pasteur's work on fowl cholera the group name of Pasteurella. He described the characters to which all members of the group conform as follows: 'An ovoid-shaped rod, polymorphous, with a special tendency towards involution forms, which is Gram-negative and exhibits bipolar staining; it is non-motile; does not liquefy gelatin; does not alter milk; does not grow on normally acid potato; does not redden Wurtz agar, i.e. does not ferment lactose; has a characteristic odour; does not produce indole in pancreatic broth; and occasions disease in animals of a septicæmic hæmorrhagic nature'. As members of this group he recognized six types isolated from (1) birds and rabbits; (2) swine; (3) sheep and goats; (4) cattle and buffaloes; (5) horses; (6) dogs. Just as he had founded the Salmonella group with *B. suipestifer* as its prototype, so he based the Pasteurella group on *B. suisepticus* as its prototype.

Later history. Investigation since then has established the fact that similar organisms occur throughout the whole animal kingdom, though Lignières's belief in the extent of their ætiological significance is no longer

shared by modern workers. The role of the pasteurella organism in certain diseases is considered to be that of a forerunner or an invader secondary to the virus actually responsible for the disease.

Pasteurellas in Man.

In contradistinction to the animal world where such virulent and widespread epizootics occur, man may be considered immune. In spite of man's close association with animal outbreaks and even of his occasionally consuming the flesh of diseased animals, very few human cases have been reported, and amongst these the diagnosis is sometimes doubtful because of the lack of a detailed bacteriological examination. The following references are to those cases where the organisms described could possibly be placed in the pasteurella group.

v. Boèr (1917) gave an account of an organism isolated from the fæces of a man who had been working among infected hens, and had acquired an acute intestinal infection. The organism was pathogenic for rabbits and pigeons and was diagnosed on the strength of its morphology and the post-mortem appearances of the animals. Commes (1918) described his bacillus as a non-motile, Gram-negative coccobacillus, which did not ferment lactose, did not alter milk, did not liquefy gelatin, did not grow on potato and was pathogenic for rabbits. He isolated it from intramuscular abscesses in four cases in Senegal. Bouffard (1920) isolated from five similar cases of pyomyositis, also in Senegal, a Gram-negative, non-motile organism which showed bipolar staining, did not grow on potato and was pathogenic for the rabbit, dog and monkey. Ortscheit (1921) described a Gram-negative, non-motile bacillus that did not liquefy gelatin and produced indole ; it was pathogenic for guinea-pigs and rabbits. He isolated it from an empyema of a boy, and it was agglutinated by the patient's serum 1/3,000.

Debré (1919) gave a much fuller account of the organism which he isolated from a purulent pleural fluid, and later from the pharynx as a saprophyte ; it can quite fairly be termed a pasteurella, being a Gram-negative, non-motile bacillus, which produced indole, gave acid in dextrose, mannitol, saccharose and lævulose, but did not ferment lactose or maltose and was pathogenic for the guinea-pig, mouse, rabbit and canary ; it agglutinated to 1/200 with the patient's serum. Teissier *et al.* (1922) also isolated an organism which probably belonged to the pasteurella group ; it came from the pleural fluid of a woman, dead after pleurisy ; it fermented dextrose and saccharose but not lactose, mannitol, maltose, lævulose (!), inulin, dulcitol or glycerol ; it produced indole and did not alter milk ; it was pathogenic for the pigeon, mouse, guinea-pig, rabbit, dog and rat.

Definition of Characters.

In the course of time the character of the organisms comprising the group has been more clearly defined, but, with the exception of indole production, the hard and fast limits laid down by Lignières have been

maintained, and the divergence in respect of this character may well be occasioned by a difference in technique involved in the test (Fazakas, 1926). To form a conception of the trend of modern opinion with regard to the cultural characters possessed by the pasteurella group in addition to those already described by Lignières, the results have been tabulated of a series of studies by 19 workers during the years 1908 to 1926 : namely Schirop (1908), Vourloud (1908), Cleland (1911), Hadley (1912), Brandt (1914), Besemer (1917), Roderick (1922), Laux (1922), Jones (1921–2), Bushnell (1923), Fitch and Nelson (1923), Gochenour (1924), Patton (1926), Meyer and Batchelder (1926), Czontos (1926), Frohböse (1926) and Tanaka (1926). The strains dealt with were isolated from animals subject to epizootics, e.g. birds, cattle, rabbits, swine, sheep.

It was soon found in the investigations referred to that in spite of the conflicting reports of earlier workers, Lignières was right in regarding the group as one of lactose non-fermenters ; the exceptions were a small minority (out of 242 only 12 strains produced acid in lactose) ; they had been classified as pasteurellas probably on the strength of the morbid and post-mortem appearances presented by the animals from which they had been isolated. Excluding these, 230 strains are left having the following characters in addition to those catalogued by Lignières.

TABLE I.

Two hundred and thirty Pasteurella strains isolated from cattle, reindeer, buffaloes, sheep, pigs, cats, hens, rabbits and rats, and described by 17 authors during the years 1908 to 1926. The figures represent the number of strains that were reported to conform, followed in brackets by that of those, if any, which did not.

Fermentations.

Acid in	*No acid in*	
Dextrose 230.	Lactose 230.	Inulin 62.
Mannitol 182 (23).	Dulcitol 73 (1).	Glycerol 32.
Saccharose 213 (3).	Arabinose 78 (25).**	Salicin 50 (15).
Lævulose 94 (4).*	Amygdalin 8 (1).	Erythritol 33.
Sorbitol 27.	Maltose 96 (17 late +).***	
Galactose 97.	Raffinose 63.	
Mannose 58.	Rhamnose 101.	
Xylose 33 (16).	Adonitol 35.	
Trehalose 15 (6).	Dextrin 62.	

* All four exceptions reported by one observer (Gochenour, 1924).
** All exceptions but one reported by one observer (Patton, 1926).
*** All 17 exceptions reported by one observer (Tanaka, 1926).

Indole Production.

Positive .. 146 Doubtful .. 1 Negative .. 12.

It will be seen that there is a general similarity between the characters of these strains except with regard to the fermentations of 5 or 6 of the sugars, namely, mannitol, salicin, xylose, trehalose and possibly arabinose and maltose.

Hassanein and Schütze (1927), examining 19 strains, derived from birds, pigs, cattle, sheep, rabbits and mice, obtained fermentations similar to those just summarized, viz. :

Acid in—dextrose, mannitol, saccharose, lævulose, sorbitol, galactose and xylose.

No acid in—lactose, dulcitol, arabinose, amygdalin, maltose, raffinose, adonitol, dextrin, inulin, glycerol, salicin, erythritol and inositol.

There were exceptions on 3 sugars ; mannitol was not fermented by 1 *boviseptica* and 1 *lepiseptica* strain ; dulcitol was fermented by 1 *lepiseptica* strain, and arabinose was fermented by 1 *boviseptica* strain. All 19 produced indole in 4 days.

Classification of Types.

On account of the similarity of their morphological and cultural characters, members of the Pasteurella group have been regarded by some as constituting a single species which has only suffered slight modifications, chiefly in respect of virulence, by prolonged existence in one species of animal or another. Other workers, however, have considered the various types to be more distinct and have recognized the necessity of raising the group to the status of a genus.

A zoological classification, i.e. a division of the group according to the animal origin of each individual strain, is the most obvious method, and has been generally adopted. It faces us with a long series of names such as *P. caviseptica, P. equiseptica, P. feliseptica*, &c., according to whether the organism has been isolated from a guinea-pig, a horse, a cat or what not. It takes for granted a homogeneity of strains within the animal species and a heterogeneity beyond it.

A classification based on morphological and cultural features is impossible. Such features only serve to establish the identity of the group. What divergence there may be in the fermentation of sugars, for instance, does not serve as a basis, and cannot be brought into line with the animal origin of the strains. To take the case of the mannitol fermenters as recorded in Table I, the non-fermenting strains were derived from seven different species of animals, the fermenting strains from all those with two other species in addition. And much the same diversity was apparent in the origin of the strains, typical and atypical, on other sugars.

A serological classification has been attempted by a number of workers employing agglutination, absorption, complement fixation, &c. ; but so far none of these methods has given a satisfactory grouping of the various

types. Chamberland and Jouan (1906) found that their attempts based on agglutination came to grief because of the ease with which strains lost their agglutinability.

Matsuda (1910) employed complement fixation and compared 6 pig, 5 fowl, 3 rabbit and 2 calf strains. When he used sera taken after only two inoculations, he was able to differentiate the zoological types; homologous fixation, i.e. within the type, was more complete than heterologous and all heterologous types fixed complement equally badly. When he used sera taken after several inoculations, there was sufficient cross-fixation to confuse the types.

Miessner and Schern (1910) were unable to show any agglutinatory relationship between *P. oviseptica* and the other pasteurellas. Bukofzer (1922) found that *P. aviseptica* strains fixed complement in the presence of *P. aviseptica* sera, but he was undecided how constant this might be for all such strains. Roderick (1922) thought that pasteurellas could by complement fixation be divided into two primary groups: (1) containing *P. boviseptica* and *P. suiseptica*; (2) containing *P. oviseptica*, *P. aviseptica*, *P. lepiseptica* and *P. caviseptica*. Jones (1921–2) divided eight typical pasteurellas isolated from pneumonias in cows and calves into two groups according to whether they fermented mannitol or not. A serum prepared with one member of a group agglutinated only its own group members. Fitch and Nelson (1923) compared the agglutination results of some 28 strains isolated from the ox, pig, sheep and fowl. They found that the serological grouping cut across the zoological. Gräfe (1923) was able to perform the Pfeiffer test with an *aviseptica* serum and strain in the peritoneal cavity of a guinea-pig, but could not group strains by this method. Niimi (1924) found cross-agglutination between the zoological types very complicated. Tanaka (1926), using complement fixation as well as agglutination, obtained results very similar to those of Fitch and Nelson, that is to say, while the average obtained for each type was highest with its homologous serum, variation within the type was great. Meyer and Batchelder (1926) found cross-agglutination to titre limit between the serum for a rat strain and an organism isolated from a hen. Lal (1927) compared the complement-fixation properties of five animal types, and found cross-fixation between all types except the *lepiseptica* one. Cornelius (1928) examined 26 strains; by absorption tests he was able to group 17 of these—Group I, 7 strains; Group II, 5 strains; Group III, 3 strains; Group IV, 2 strains; the serological classification bore no relationship to the zoological derivation of the strains.

Agreement has obviously not been arrived at; for example, while Miessner and Schern found *aviseptica* serologically unrelated to other types, Fitch and Tanaka both thought it had close affinity with *boviseptica*, whereas Roderick regarded it as akin to *oviseptica*, *caviseptica* and *lepiseptica*, and Lal would ally it with *suiseptica*.

A classification by pathogenicity is similarly impracticable; for, as Chamberland and Jouan (1906) have stated, although very considerable

specificity in virulence is shown by the various zoological types, cross-infection is possible to a notable degree. Hueppe (1886) showed that *boviseptica* was virulent for pigs, goats, rabbits, mice and pigeons. Kemény (1925) saw a pasteurella outbreak pass from buffaloes to pigs. Voges (1896) said that *P. suiseptica* could spontaneously infect hens. Scennikov (1926) described pasteurellosis among hens after they had been fed with the blood of a pig dead of a *suiseptica* infection. On the other hand, Georges (1904) saw pigs succumb to *aviseptica* strains. Bruynoghe (1914) infected guinea-pigs, rats and mice with *lepiseptica*. Haan (1920) saw pigs go down with swine-fever after being let into an undisinfected stall which had held sheep suffering from pasteurellosis.

A classification by immunity was thought by Lignières (1906) to be an easy method for the differentiation of the various types. For example, the organism which protects an animal against a virulent *P. suiseptica* must be a *suiseptica* itself, and so on ; but most workers are agreed that although homologous immunity is more readily achieved than heterologous, cross-immunity is possible. Chamberland and Jouan (1906) induced immunity to *aviseptica* by inoculating the fowls with either *lepiseptica* or *equiseptica*, though Miessner and Schern (1910) could not confirm this. Gallagher (1916) protected rabbits against *suiseptica* with *aviseptica* inoculations, while Meyer and Batchelder (1926) rendered guinea-pigs and rats immune to a rat pasteurella by inoculating with an *aviseptica*. When one recalls how difficult and uncertain the immunization of animals against pasteurellas is, the existence to this extent of cross-immunization robs classification by immunity of its plausibility.

As none of these methods of classification is a reliable means of dividing the Pasteurellas into types, and until such time as definite results demonstrate the existence of clearly marked varieties within the group, the Pasteurellas should be regarded as a collection of strains fundamentally identical, though capable of adaptation in respect of one or other animal host. However, for the sake of convenience in the discussion of work done on the various pasteurelloses, the subject-matter will be segregated under headings corresponding to the animals concerned. Such a segregation must be clearly recognized as purely arbitrary, and as possibly concealing real differences and creating fictitious ones.

Characters of the Pasteurella Group.

As no cultural characterization serves to distinguish one of these animal types from another, the description of the group as a whole will be given here and held to apply to each of the individual members.

The organisms are short oval cells, 0·3 to 1·25μ in length, occurring as single cells or in pairs, rarely in chains. In lightly stained preparations the ends of the rod are seen to take the dye more definitely than the central portion. This bipolar staining may be missed in smears made from cultures, and is always more pronounced in bacteria derived directly from the body. Spores are not formed, and the organisms are non-motile

and without flagella. Capsules are usually regarded as not being present, though Carpano (1913) thought that he had demonstrated them by special fixation methods, as did Gózony (1913), by means of Indian ink preparations. Hadley, Bryant and Elkins (1914) could not confirm Gózony's findings and regarded his encapsuled bacteria as not being pasteurellas. Manninger (1919) described capsules for his virulent but not for his avirulent variant of *P. aviseptica*. The Pasteurella group stains with the usual dyes and is Gram-negative.

Growth takes place more readily under aerobic than anaerobic conditions. The optimum temperature is 37° C. Good growth is obtained on all ordinary media, except in the case of a very small seeding, when, as Webster (1925) has shown, some accessory substance which apparently regulates oxygen tension and has been demonstrated to exist in blood, is needed. Webster demonstrated that a seeding of one bacillus would result in growth in a tube of broth when the admission of air was impeded by a vaseline seal or when rabbit's blood in an amount as small as 0·0003 per cent. had been added, while in an open tube of ordinary broth it needed as many as 100,000 organisms before growth would result. Schütze and Hassanein (1927) obtained similar results, using solid media. A seeding, for instance, that was too small to give any colonies on an agar plate, would result in a plentiful supply when the agar was not poured, but was left in the form of an agar shake, with a consequently lowered air supply. An agar plate to which a small amount of blood had been added would grow, for example, more than 7,000 colonies from a seeding that gave no colonies on an ordinary agar plate. Not every strain possesses the same degree of oxygen sensitiveness and will give such striking results. Webster and Baudisch (1925) substituted certain inorganic iron compounds such as Fe_3O_4, in the place of blood and they had the same augmenting effect on growth ; these substances possessed oxygen-absorbing properties.

Harvey and Iyengar (1921–2) have shown that grown on blood-agar and kept sealed, a culture will remain alive for at least a year.

Growth in broth occurs with almost normal rapidity as a delicate general turbidity and on agar as a fine translucent growth. It is accompanied by a characteristic odour. No visible growth takes place on potato. Bile salts inhibit the growth of pasteurellas, MacConkey's medium being used to differentiate them from *B. pestis*, which grows in the presence of such salts.

Gelatin is not liquefied, milk remains unaltered in reaction and is not coagulated, nitrates are reduced to nitrites, indole is considered by nearly all modern investigators to be produced, and also H_2S.

The most generally accepted sugar fermentations are : *Acid* in glucose, mannitol (with some exceptions), saccharose, lævulose, sorbitol, galactose, mannose, xylose and trehalose. (Both the latter with exceptions.) *No acid* in lactose, dulcitol, arabinose (with exceptions), amygdalin, maltose (with exceptions), raffinose, rhamnose, adonitol, dextrin, inulin, glycerol, salicin (with exceptions) and erythritol. No gas is ever produced.

A variant form has been described by De Kruif (1921[1]) which has slightly different cultural characters. It is probably biologically equivalent to the ' rough ' variant of the Salmonellas, *B. dysenteriæ*, &c. On agar the growth is more opaque. In broth it is granular, and sinks to the bottom of the tube, leaving the supernatant fluid perfectly clear. The variant form is, therefore, designated G (granular) in opposition to the normal which is named D (diffuse). Webster (1925) found that this G variant is not sensitive to oxygen pressure. Light seedings can, therefore, produce growth without the addition of any accessory substance such as is necessary for the normal D form.

Resistance of pasteurellas to lethal influences is slight ; drying, sunlight, heating above 45° C. and disinfectants easily destroy them. Protected from unfavourable influences viability is maintained for long periods. Holmes (1914) found that cultures mixed with unsterilized mud and protected from direct sunlight remained alive for at least three months. It is thus very likely that the organism is widely spread as a temporary saprophyte, during, and in the neighbourhood of, an epizootic, and can, under suitable circumstances, maintain itself there for some months at least.

In the course of acute disease the bacillus can be isolated from the blood and internal organs, as well as from the excretions ; in chronic forms it may be restricted to some special region, the pleuræ, intestinal tract, &c. That carriers exist has been shown by the fact that pasteurellas have been frequently isolated from the mucous membranes of healthy animals, both in virulent and avirulent forms (Smith, 1891, 1892 ; Koske, 1905 ; Klein, 1906 ; De Kruif, 1922[2] ; Webster, 1924 ; Smith, 1927).

Variation in virulence is a notable feature among pasteurella strains. To preserve virulence, growth on blood-agar is recommended by Harvey and Iyengar (1921–2, 1922–3, 1923) ; if the culture is kept sealed its virulence is maintained for at least a year. They considered that animal passage, but not frequent subculture, even on a favourable medium, will raise virulence.

Differential Diagnosis.

Two bacterial species, *B. pestis* and *B. pseudotuberculosis rodentium*, to which the pasteurellas are most nearly related, should be mentioned here, as their similarities and differences are chiefly concerned with the characters that have just been recorded. The similarity between *B. pestis* and the pasteurellas in morphology, bipolar staining property, type of growth on ordinary media, and pathogenic action in the bodies of those rodents where both can be met with, may lead to a confusion between the two organisms, but they are to be differentiated by (1) their fermentation of sugars ; (2) indole and H_2S production ; (3) growth in the presence of bile salts ; (4) the character of the agar colonies, Pasteurella giving delicate even-edged forms of a fine ground glass appearance, and *B. pestis* coarsely granular forms with crenellated margins, though occasionally

less irregular shapes are seen ; (5) *B. pestis* displays marked virulence for the guinea-pig ; rubbed into the shaven belly, a virulent strain will, unlike Pasteurella, occasion rapid death. (See Table II.)

The differentiation of *B. pseudotuberculosis rodentium* and the pasteurella group is based on the following features possessed by the former. Morphologically it is a larger, more rod-like organism, it grows more heavily on ordinary media, possesses flagella and at temperatures below 30° exhibits motility (Arkwright, 1927), does not produce indole or H_2S and turns litmus milk alkaline. Otten (1926) has described a medium, consisting of 0·05 per cent. glucose in 0·5 per cent. peptone water which is said to differentiate the three organisms from one another. Litmus serves as an indicator. *B. pestis* turns the medium acid within 24 hours ; pasteurella does so only after 2 to 3 days ; the acidity remains in both cases. *B. pseudotuberculosis* produces acid rapidly, but within 3 days the medium has reverted to neutrality and by the seventh day is alkaline.

For differences in the fermentation of sugars, see Table II.

Pathogenic Action.

Pathogenicity is a noticeably variable feature in members of the pasteurella group. Virulent organisms entering the body of a susceptible animal can multiply with such rapidity in the tissues and blood that they occasion death in a few hours. D'Herelle (1926) describes the startling suddenness of the disease in buffaloes, where the first indication may be that the yoked animal comes to a standstill and after a moment of distress falls to the ground as if struck by lightning. Hens are found dead in the nest, and on, or under, the roosts, without previously showing signs of illness. The amount needed to infect and occasion the death of a susceptible animal can be very small. Acute forms of the disease produce a generalized hæmorrhagic inflammation accompanied by high fever ; blood-stained exudates collect in the serous cavities, and the connective tissue spaces may be filled with large amounts of these fluids ; punctiform hæmorrhages under the serous and mucous membranes are a constant accompaniment. More chronic forms of the disease result in more localized reactions ; the lungs are frequently involved and abscess formation may result at the site of inoculation.

The toxic substances which effect these destructive processes are little understood. Pasteur (1880[1 & 2]) was the first to announce a soluble toxin existing in filtered broth cultures of *P. aviseptica*. This filtrate had a narcotic effect on hens which soon wore off without doing further damage to the animals. Klett (1904) reported that similar effects could be produced in hens by the injection of filtered broth cultures of *suiseptica*. Other workers, Voges (1896) and Hadley (1918), regarded these toxic effects as due to endotoxins liberated during the autolysis of old cultures. Filtrates of young broth cultures contain no toxins ; on the other hand, the bodies of the bacilli themselves yield markedly toxic substances. With these derived from *suiseptica*, Macfadyen (1907) was

TABLE II.

	Growth in bile salt media	Indole and H_2S	Motility	Litmus Milk	Rhamnose	Glycerol	Saccharose	Sorbitol
B. pestis	+	−	−	Neutral	+	±	−	−
Pasteurella	−	+	−	Neutral	−	−	+	+
B. pseudotuberculosis	+	−	+ below 30° C.	Alkaline	+	+	−	−

able to cause in the usual laboratory animals stupor, diarrhœa, œdema, hæmorrhages and death. Just as strains vary in virulence so do they in toxicity, but the two characters are not dependent one on the other (Klett, 1904).

In contradistinction to most authors Calamida (1903) reported the existence of hæmolysins.

In a series of papers Weil (1905[1 & 2], 1907, 1908) developed Bail's theory of aggressins in respect of *P. aviseptica*, and combated the contention of Citron and Pütz (1907). Citron and Pütz (1907) thought that similar substances could be obtained by merely washing cultures of *P. aviseptica* with saline or serum ; these extracts they called artificial aggressins.

The route of infection is considered to be a direct one through some break in the continuity of surface of skin or mucous membrane. The bacteria are capable of surviving for long periods in situations where they are sheltered from the lethal effects of light and drought. Healthy animal carriers of virulent as well as avirulent organisms are known to exist, and they probably possess some immunity, as the serum of such animals, according to De Kruif (1922[2]), contains immune bodies which do not occur in the serum of animals not carrying the germ. He demonstrated complete agglutination of *P. lepiseptica* in 7 out of 9 rabbits, with titres ranging as high as 1/80, when the serum was derived from animals carrying that organism on their nasal mucous membranes ; there was in no case complete agglutination, even in dilutions of 1/10, among the 24 rabbits tested in whose nasal passages no pasteurella had been detected. This immunity would seem to be a relative one and to be overcome upon exposure to organisms of still greater virulence (Smith, 1927). No insect vectors of the disease are known ; so it happens that outbreaks may remain confined to certain aggregates of animals, separated from neighbouring healthy ones merely by wire fencing.

The pasteurella organism is at times the sole cause of the morbid process with which it is associated ; at other times it may be in partnership. Webster (1924) and Bull and McKee (1927) found *P. lepiseptica* and *B. bronchisepticus* acting jointly in the production of snuffles in rabbits. And sometimes pasteurellas are regarded as mere secondary invaders ; an opinion held by Müller and Schmid (1919), Hegyeli (1921), and Schalk and Roderick (1922) in the case of swine plague. The association of pasteurellas with such diseases as canine distemper and equine influenza may be regarded as a purely secondary one.

The Zoological Types.

As already indicated, the pasteurellas have been regarded as the cause of disease in almost every species of the animal world. The following may be regarded as the more important types, *P. aviseptica*, *P. suiseptica*, *P. boviseptica*, *P. oviseptica* and *P. lepiseptica*, and these types will be considered in the following pages. Some more recent references to the

occurrence of pasteurelloses in other animals may be given here. Crows and ravens (Cernaianu, 1925); ferrets (Kister and Schmidt, 1904); rats (Meyer and Batchelder, 1926; Smillie, 1920); reindeer (Magnusson, 1913; Brandt, 1914; Horne, 1915; Grüner, 1926); elephants (Mitra, 1914); cats (Pospischil, 1920; Frohböse, 1926); donkeys (Schmid, 1920); horses and mules (Nickewitsch, 1914; Hardenbergh and Boerner, 1917; Patterson, 1919; Timčenko and Moskalev, 1925); camels (Cross, 1917, 1919; Littlewood, 1920); guinea-pigs (Freund, 1926; Reed and Ettinger, 1927).

Pasteurella aviseptica.

Synonyms. B. choleræ gallinarum, P. avium, B. avicidum.

The bacillus of fowl cholera.

History.

The organism was first described by Perroncito (1878) and Toussaint (1879). Pasteur (1880[1 & 2]) isolated and studied it.

Pathogenicity.

The organism is pathogenic for practically all the domesticated and wild varieties of birds. Experimentally it can cause disease in guinea-pigs, rats, rabbits, mice, pigs, cats, sheep and even cattle and horses. The degree of infectivity varies in different animals; it is, for example, marked for birds and rabbits, but very much less so for guinea-pigs, unless given intraperitoneally.

Pathogenic Action.

A virulent strain produces a fatal septicæmic infection; death may occur within 24 hours and the mortality reach over 90 per cent. Diarrhœa is a noticeable accompaniment. The post-mortem appearances are those of general engorgement of the blood-vessels with ecchymoses in the heart-walls and the intestinal tract. A fibrin-flecked fluid collects in the pericardial cavity. The bacterium is present in the droppings as well as the blood and tissues of the affected bird. A less virulent strain may occasion only diarrhœa, wasting and an arthritic involvement of the joints. Seddon (1914) described an outbreak in the neighbourhood of Melbourne, Australia, occasioning œdema in the wattles of fowls, the morbid process being usually confined to those parts. Jackley (1917), Bushnell (1923), and Weaver and Mitchell (1924) consider chicken roup to be caused by *P. aviseptica*. Jackley found no organisms in the cultural examination of the blood and viscera of fowls ill with roup, but in the early stages of this disease he isolated *P. aviseptica* from the eye lesions. Inoculation with this bacillus reproduced the disease. Bushnell compared strains isolated from eye lesions in roup with *P. aviseptica* and found them identical, but he could not determine why in one case roup and in another cholera was caused by the same organism. Weaver and Mitchell isolated *P. aviseptica* from roup in fowls, and could induce this disease by inoculation with the isolated bacillus.

Ingestion has been noticed to be an unreliable method of causing infection in hens unless the food is supplied in liquid form, in which case it is probable that owing to the existence of a naturally cleft palate, infection is a nasal one. Staub's (1925) experiments point to a rapid loss of virulence where infection is attempted by feeding the intestinal contents of one bird dead of the diseases to a second, and so on.

Death is due to toxins liberated by bacteria, probably as their lysis takes place. No evidence of exotoxins capable of causing death has been brought forward. Hadley (1918) considers Bull (1916) to have been dealing with fowl typhoid and not fowl cholera, and that, therefore, his description of soluble toxins does not apply. Pasteur (1880[1 & 2]) and others have demonstrated an exotoxin, but its effect on hens was merely a narcotic one.

The variation in toxicity of different strains is very great. Cultures have been described of such virulence that apparently a single organism is capable of causing the death of a bird. On the other hand, avirulent races exist, of which many million bacteria may be inoculated without ill effects. Calamida (1903) is the only worker who has claimed to have demonstrated the existence of hæmolysins in *aviseptica* cultures. Aggressins are stated by Weil (1905[1]) to be present in the exudates of animals inoculated with the organism.

Diagnosis.

The organism can be isolated from the droppings, or whatever morbid products of the living bird may be available, and from the blood and tissues of dead animals. Pegreffi (1926) recommends the scarification of the conjunctival sac of a healthy fowl and the rubbing in of the suspected material. Even if there is gross contamination the method succeeds and the bird dies within 16 to 18 hours.

Immunization.

With a living inoculum. The first attempts at prophylactic inoculation were made by Pasteur (1880[1 & 2]) in his experiments with attenuated living vaccines, and in making them he laid the foundation of the whole study of prophylactic inoculation. He found that old broth cultures, by reason of their exposure to air, decreased in virulence. By inoculating such attenuated cultures, he claimed to be able to establish immunity against virulent strains of fowl cholera. Subsequent workers, Kitt (1886), Cagny (1885), Hess (1886), were, however, able to show that not only was the method a dangerous one, but complete protection against subsequent inoculation with virulent bacilli could not be relied upon.

Lignières (1902) still advocated the use of living vaccine prepared from strains that had been subcultured on agar more than 500 times and had thus lost all virulence. He recommended a multivalent vaccine prepared from all pasteurella types. Even so he only achieved a relative protection, the mortality among the inoculated being 12 to 15 per cent. as compared

with 50 per cent. among the uninoculated. Chamberland and Jouan (1906), regarding the pasteurella group as consisting essentially of a single variety, considered that one type could protect against another. They inoculated with living *suiseptica*, *lepiseptica* or *equiseptica* strains, but were only able to protect hens against *P. aviseptica* in a proportion of cases. Hadley (1912, 1913, 1914[1 & 2]) found that one but only one of his 16 avirulent strains used in the form of a living vaccine was capable of immunizing against highly virulent cultures. This strain differed from the others in its power of attacking saccharose (Hadley *et al.*, 1919). Most of Hadley's work was done on rabbits. Subcutaneous injection of 0·0001 to 3 c.cm. of a broth culture of this strain generally produced a marked inflammatory local reaction followed by abscess formation. After 7 days, the rabbit could be shown to be immune to 2 c.cm. of a culture which killed controls in 12 hours in such minute doses as one thousand millionth of a c.cm. This immunity appeared to last for years and to be communicable even after two years in a passive form by a female animal to its offspring. Three young rabbits whose mothers had been immunized two and a quarter years previously, all survived inoculation with 0·000,01 c.cm. of a virulent culture, which represented many thousand m.l.d. ; that this passive immunity was not as solid as the active one of the parents was shown by the fact that only 2 out of 10 offspring, born 1 to 2 years after the immunization of their parents, survived the larger dose of 0·001 c.cm. Passive immunity would seem to last about 40 days, for 5 rabbits tested when 30, 32, 32, 35 and 40 days old, all survived the test doses, while 1 which was 44 days old succumbed. In pigeons and fowls the results were not so reliable.

Gallagher (1916) repeated Hadley's work and found that this avirulent strain when inoculated living, immunized fowls, rabbits and guinea-pigs against *P. aviseptica*, but not against the highest doses. Manninger (1919, 1921) plated an old fowl cholera culture and picked atypical, opaque, brown colonies that proved to be avirulent. A living vaccine prepared from agar cultures and given in 5 mgm. doses to hens never caused death as Pasteur's inoculation did. He recorded protection in mice, pigeons and hens.

Szász (1919) gave a single dose of a vaccine which was a 4 to 5 day broth culture of virulent *aviseptica*, containing no, or few, active bacteria. Sometimes the reaction was marked, but rarely dangerous. He considered that he could lower mortality from 34 to less than 3 per cent. Grimm and Pfeiler (1919) thought that the reactions obtained with Szász's vaccine were too severe and that the results of Neumann's (1919) and Schreiber and Stickdorn's (1918) inoculations were too unreliable. Schreiber and Stickdorn used a vaccine containing large numbers of dead *aviseptica* and their products of metabolism, but tested it only in the field, because of the great virulence of their laboratory strains. They reported the successful use of this vaccine in combating epizootics. Sakamoto (1922) had no satisfactory results in his attempts to immunize pigeons against

fowl cholera, using a vaccine attenuated by incubation at 42 to 43° C. for 10 days. Staub (1925) advised giving a preliminary inoculation of living *P. lepiseptica*, which is non-pathogenic for fowls and produces a temporary immunity. He followed this up with an attenuated Pasteurian vaccine which was then, he said, not dangerous and gave complete immunity. Laboratory methods of testing for immunity he considered unsatisfactory, not reproducing the conditions met with in nature.

Broudin (1926) isolated from the marrow of the tarsus of a hen dead of fowl cholera a bacteriophage specific for *P. aviseptica*. It would not act on *P. boviseptica*, just as D'Herelle's (1921) bacteriophage from the buffalo would not act on *P. aviseptica*. Birds of all kinds inoculated with $\frac{1}{2}$ to 10 drops of this *P. aviseptica* bacteriophage were reported to be immune to an *aviseptica* infection.

The difficulty that has been experienced in immunizing hens against fowl cholera is demonstrated by this list of conflicting reports, and the danger involved in employing a living inoculum is evident. The degree of protection that may have resulted is usually only put to the test of natural infection in the field, so that it is generally impossible to form an accurate idea as to its extent. With rabbits, Hadley has reported a striking series of immunizations, and a surprising individuality in strains for vaccine purposes was noticed. Only with one of his strains was he able to immunize, but with this avirulent organism he succeeded in conferring solid immunity against very virulent cultures.

Immunization with a killed inoculum. Weil (1905[1 & 2], 1907, 1908) applied to fowl cholera Bail's aggressin principles. He demonstrated that the pleural exudate sterilized at 44° C. with 0·5 per cent. phenol of rabbits dead of an *aviseptica* infection, while being in itself innocuous, contained substances, the so-called aggressins, which increased the infective power of an inoculation with living *aviseptica*. If a sterilized exudate is given prophylactically anti-aggressins are produced and these render the animal immune to *P. aviseptica*. Citron and Pütz (1907) prepared what they called artificial aggressins, by extracting cultures with water or serum, and claimed to be able to protect rabbits and pigeons against fowl cholera. Weil considered that these workers had only established a partial immunity, and pointed out the smallness of their test doses. He would not accept their artificial aggressins as similar to his natural aggressins either in their nature or effect. Huntemüller (1906) could protect rabbits against *P. aviseptica* by inoculating with either exudate or agar-grown cultures killed by treating with 0·5 per cent. phenol for 3 hours at 44° C., and for 7 days at 37° C. When filtered through a Berkefeld filter these preparations did not protect. He, therefore, found himself in agreement with Citron and Pütz and in opposition to Weil's theory, and regarded the immunizing substance as localized in the body of the bacillus.

Telitschenko (1911) was unable to protect fowls with *aviseptica* vaccines, killed at 60° C., even with as many as three inoculations.

Hadley and Caldwell (1919) used a killed vaccine made with the avirulent strain which Hadley had found so effective when inoculated living. Even after as many as 5 doses protection was unlikely with fowls, only 1 out of 9 surviving a test dose which killed controls not more quickly than in 42 and 44 hours. Rabbits could be protected provided that they received sufficient inoculum ; four animals receiving 2 to 5 doses of 2 c.cm. vaccine survived a test dose which killed the control in 13 hours, but a single inoculation of 2 c.cm. or two of 1 c.cm. failed to protect two other rabbits. Vaccines of virulent strains prepared in the same way, i.e. 48-hour broth cultures heated at 63° C. for 30 minutes, were no better. Gallagher (1916) used Hadley's avirulent strain, but whether killed at 60° C., by phenol or by ether, it failed to immunize. Jackley (1917) considered that a 4-day-old broth culture killed without heat by the addition of 0·5 per cent. phenol, was able to protect fowls. In a series of papers, Harvey and Iyengar (1921–2, 1922–3, 1923) and Iyengar alone (1925) tested the efficacy of heat-killed *aviseptica* vaccines administered to pigeons by the intravenous route. With vaccines made from either virulent or avirulent strains pigeons could be protected against as many as 50 or 100 m.l.d. ; a corresponding vaccine made from a heterologous type (*P. lepiseptica*) or an altogether alien species (*B. coli*) protected only against 1 or 2 m.l.d. The prophylactic dose giving the best result lay between 0·01 and 1 mgm. ; higher doses appeared to influence adversely the response to inoculation, and lower ones were insufficient to produce maximum immunity. Vaccines prepared by heating to 40 or 60° C. for half an hour and adding 0·5 per cent. phenol or by killing merely with 0·5 per cent. phenol, were all equally good and much superior to those heated to 80° C. ; with the latter immunity was low and lasted only 2 months, whereas with low temperature vaccines good immunity was maintained for 4 months.

Kept at room temperature or 37° C. vaccines do not deteriorate in 9 months.

Sakamoto (1922), using pigeons and giving one subcutaneous inoculation, tested vaccines killed by heating to 60° C., drying on slides, shaking for 24 hours or treating with iodine, but obtained only unsatisfactory results, the dose being 1/10 to 1/50 of an agar slope. However, when given 2/3 of an agar slope of a mixed fowl, rabbit, swine and guinea-pig pasteurella vaccine, agar-grown and killed by heating at 60° C. for 1 hour, pigeons resisted 100 m.l.d. of *P. aviseptica*, and 1 c.cm. of a concentrated 11-day broth culture of *P. aviseptica*, i.e. evaporated in a vacuum for 4 hours at 45 to 50° C. to 1/10 of its volume, protected against 10 m.l.d. Weil's use of aggressins was tested by heating for 3 hours at 44° C. the pleural exudate of a rabbit dead after an *aviseptica* inoculation, passing it through a Berkefeld filter and adding 0·5 per cent. phenol. A ½ c.cm. of this would protect against a lethal dose of the virulent *aviseptica*. In thus finding filtered exudate potent, Sakamoto is in opposition to Huntemüller (1906) but in agreement with Gochenour (1924) who worked with

P. boviseptica. Finally, with nucleoproteids prepared according to Lustig-Galleotti (48-hour agar cultures treated with 1 per cent. KOH at room temperature, neutralized with 1 per cent. acetic acid, centrifuged and washed with distilled water and dissolved in 1 per cent. sodium carbonate) and made from mixed cultures of swine, rabbit, guinea-pig and fowl bacilli, giving a dose corresponding to 2/3 of a slope, Sakamoto could protect against 1,000 m.l.d. Unlike most of the other vaccines, which occasioned abscess formation at the site of inoculation, the nucleoproteids produced no ill-effects. Vigadi (1924) attempted to immunize hens with broth culture killed at 55 to 75° C. Even 4 doses of 2 c.cm. each only resulted in death being delayed for 1 to 10 days after the controls. Cernaianu (1924, 1925) inoculated fowls and rabbits with vaccine heated to 60° C. for 1 hour. The hens were tested against natural infection in the field. He reported that the epizootic always came to an end 4 to 5 days after inoculation and that immunity lasted 3 to 5 months. The inoculated rabbits were tested against small subcutaneous injections of *P. aviseptica* and found to survive, if not more than 2 or 3 m.l.d. were given. A multivalent vaccine of newly isolated virulent strains is recommended. Scennikov (1926) failed to protect fowls with vaccines killed with heat or phenol, treated with alcohol, or with natural and artificial aggressins.

The work of most investigators makes it apparent that killed inocula do not produce in hens an immunity sufficiently solid to withstand laboratory infections, and the evidence of protection in the field is by no means convincing. In the case of pigeons the results are more satisfactory and Harvey and Iyengar as well as Sakamoto achieved notable results, as the following summary of one of the experiments of Harvey and Iyengar will show.

Pigeons inoculated with two intravenous doses of 0·04 and 0·08 mgm. heat-killed vaccine were given test doses of the living organism 30 to 120 days after the second inoculation ; the results are given below :

Size of Test Dose. m.l.d.	*Number Tested*	*Number Survived*
5–25	24	24
10–50	24	24
50–250	24	23
100–500	24	18
500–2,500	24	6

A similar degree of protection was demonstrated in several experiments.

Immunization of the rabbit with killed vaccine is an easier task and has been achieved by Weil and Huntemüller and also by Hadley, who found it essential to give large and repeated inoculations.

Passive immunity. Protection by means of products derived from immunized animals was first attempted by Kitt (1892), who used the blood, tissue juice and eggs of inoculated animals. Voges (1896) and

Kitt and Mayr (1897) made use of the serum of such animals. A certain amount of protection against lethal doses was all they could report, and, as Voges showed, this degree of immunity was just as well achieved with normal serum as with that of an immune animal. He found, too, that if serum was injected at the same time as culture, death was not delayed ; the serum had to be given 24 hours in advance to achieve its effect ; it acted upon the animal itself, intensifying its powers of offence and defence, rather than on the virus introduced ; what protection did result was thus caused in a different manner from that afforded by a true immune serum. Weil (1905[2]) succeeded to some extent with anti-*aviseptica* serum in protecting mice and rabbits, but not pigeons, against *P. aviseptica*. Citron and Pütz (1907) had similar results whether they used anti-*suiseptica*, anti-*boviseptica* or anti-*aviseptica* sera. Chamberland and Jouan (1906), Skarschewskij (1911), Hadley (1912), Hansen (1912), Manninger (1921) and Busson (1921) all came to the conclusion that anti-serum can only delay death for a short time (1 to 2 weeks) ; it does not cure. Hansen saw a danger in its use ; sick poultry can be kept well sufficiently long for their sale, the disease subsequently breaking out and infecting the new farms to which they have been dispersed. Manninger thought the serum good in order to prolong the life of birds shortly due to be slaughtered for the market or in order to give time to work up an active immunity with vaccine inoculations. Busson agrees with Voges that true passive immunity, just like true active immunity, is not possible ; there is merely a non-specific raising of the animal's power of resistance. Still, this raising of resistance may be quite an important factor in the prevention of infection. The power of staving off death which serum possesses, makes it a useful adjunct to inoculation, and as such it has been used by, amongst others, Schreiber and Stickdorn (1918), Grimm and Pfeiler (1919), Pfeiler (1919, 1920), Manninger (1921) and Cernaianu (1925).

Antibacterial Measures.

No treatment of the infected animals is considered of use. To combat the spread of infection, the slaughter of sick birds and the removal of dead ones are recommended, together with other measures, such as isolation of centres of infection, disinfection of premises, and sterilization of drinking-water by the addition of 0·2 per mille mercuric chloride.

Pasteurella suiseptica.

Synonyms. *B. suicida*, *P. suis*.

The bacillus of swine plague and the contagious pneumonia of pigs.

History.

Loeffler (1886) differentiated the disease from swine erysipelas and isolated the organisms, as did Schütz (1886) and Smith (1886).

Pathogenicity.

Besides swine many other animals can be infected by inoculation with virulent strains. Mice and rabbits are very susceptible ; birds and guinea-pigs are less so, but even to cattle, horses, sheep, goats and cats the disease can be communicated.

Pathogenic Action.

The disease, which is usually sporadic in nature, may take the form of a rapid septicæmia ; the animal dies in 20 to 30 hours with all the signs of a typical hæmorrhagic septicæmia ; petechiæ under the skin, in the heart and other organs ; serous effusions in the subcutaneous tissues and thoracic cavities. The more usual chronic infections result in necrotic pneumonias with caseous patches in the lungs, and with or without pleuritis, wasting and joint affections. The bacilli in acute cases are easily recovered from the blood and internal organs, in chronic cases from the necrotic lesions with greater difficulty. The bacillus in such chronic cases is often in association with a variety of other organisms. It is also seen as a secondary invader in cases of swine fever, and it was only when a filterable virus was recognized as the ætiological factor in this disease that the true position of *P. suiseptica* was realized. This ill-understood association with another disease has occasioned much confusion in the reported symptomatology of swine plague, so much so that many authorities have declared against the existence of an independent disease in pigs due to *P. suiseptica*.

Infection by feeding is very difficult unless the general resistance of the animal has been lowered by cold or some other factor. It is probable, therefore, that, failing this preliminary weakening, pigs are naturally infected by the respiratory tract or through wounds in the skin or mucous membranes.

Free soluble toxins have not been demonstrated in broth cultures of this bacillus. Sélander (1890) and Silberschmidt (1895) described toxic effects in filtered cultures, but these were many days old and given in large quantities ; they probably contained endotoxins liberated by disintegrated bacteria such as were demonstrated by Macfadyen (1907), who, by grinding organisms at the temperature of liquid air, extracted a toxic substance that occasioned stupor, diarrhœa and hæmorrhages, and was fatal for rabbits in amounts as low as 0·5 mgm.

Diagnosis.

In acute cases the bacterium is easily isolated for purposes of diagnosis ; in more chronic ones it may be difficult to do so, and the inoculation of a pig with portions of the diseased organs may be necessary before the organism can be recovered.

Immunization.

From earlier times up to the present day investigators have differed as to the success with which animals can be immunized against

P. suiseptica infections. Among the workers reporting positive results were Schweinitz (1893), who protected guinea-pigs and swine by means of his vaccine of ' albumoses ' and ' ptomaines ' derived from *P. suiseptica* cultures ; Smith and Moore (1894), who used a vaccine killed at 58° C. and protected both rabbits and guinea-pigs ; Silberschmidt (1895), who used sterilized or filtered broth cultures of virulent strains and sterilized blood taken from an animal dead of a disease ; Lignières (1902), with living cultures of attenuated strains ; Chamberland and Jouan (1906) also with living vaccines ; Kitt (1905) ; Citron (1906) ; and Weil (1906), who, like Miessner and Schern (1910), used sterile exudates from infected animals, the aggressins of Bail. Broll (1908) tried killed vaccines of both *P. aviseptica* and *P. suiseptica*, with varying results. He found Conradi's (1903) autolysates too toxic for use.

On the other hand, negative or at best unsatisfactory immunity results have been reported by Voges (1896), using killed cultures, and Beck and Koske (1905), who devised a method consisting of a preliminary peritoneal inoculation of killed or attenuated bacilli followed by an intramuscular injection of living virulent *P. suiseptica*. Broll (1908) considered Beck and Koske's system of double inoculation too dangerous and likely to cause a spread of the disease.

Graham and Schwarze (1921), in a large series of inoculations with heat-killed vaccines, did not succeed in immunizing rabbits and guinea-pigs. Krautstrunk (1904) was able to accustom animals to gradually increasing doses of a living *P. suiseptica*. When, however, a different strain of that organism was interposed in the series of injections, the animals succumbed as speedily as the controls, even though the dose on this occasion was not raised. His inference was that the immunity produced was strictly confined to the strain actually used in the vaccine.

Passive immunity. As with active immunity so with passive immunization opinions differ as to the value of the procedure. Voges (1896) was able to protect guinea-pigs and rabbits to a certain extent, using serum derived from a sheep immunized with *P. suiseptica* and giving it 24 hours before the infecting dose. But he also found that similar protection was afforded by injections of normal serum. Lignières and Spitz (1902) advised the use of a serum produced by the inoculation of a variety of types so that it might be used in a similar variety of pasteurelloses, and regarded the product as very effective both curatively and prophylactically. Bruck (1904) found anti-*suiseptica* serum gave protection for mice, but definitely only for the homologous strain ; against another *suiseptica* strain the immunity conferred might be much lower. Chamberland and Jouan (1906) by means of their serum were able to delay the death of rabbits only for a time reckoned in hours to one reckoned in days. Citron (1906) made anti-*suiseptica* sera by inoculating with bacterial extracts of animal exudates and demonstrated some degree of protection by means of them. Later, Citron and Pütz (1907) showed that anti-*aviseptica* and anti-*boviseptica* sera could protect against *P. suiseptica*

infections to a slight extent, which conflicted with Voges's (1902) assertion that anti-*aviseptica* serum did not protect against *P. suiseptica* infection. He used Pfeiffer's experiment to demonstrate the lack of cross-protection. Haan (1920) considered it advisable to combine active with passive immunity, and gave swine-plague serum and vaccine simultaneously with good results.

It is quite obvious that no safe or reliable method of active immunization has been arrived at. No author gives definite and convincing experimental results. The best are obtained with living inocula, but always at the risk of killing the inoculated animals and of spreading infection to others. The degree of passive immunity that can be achieved is only moderate and it would seem as if similar protection is afforded by the injection of any normal serum.

Antibacterial Measures.

The maintenance of the natural resistance of the animal by the establishment of a good environment and the supply of an adequate diet is of first importance as a prophylactic measure. If serum treatment is considered, it is probably advisable to give vaccine inoculations at the same time. The segregation of already infected animals is an obvious means of attempting control of the spread of infection. A number of investigators (Martens, 1917 ; Hoffman, 1917 ; Raebiger, 1918) consider that the therapeutic administration of methylene blue is advantageous. In doses of 0·3 gm. *per os* daily it is harmless and seems beneficial. Holmes (1914) recommended iodine. Treated either *per os* or subcutaneously 16 hours after infection, 50 per cent. of the animals survived. He found potassium permanganate without effect.

Pasteurella boviseptica.

Synonyms. *P. bovis.*

The bacillus of hæmorrhagic septicæmia of cattle, deer, wild boars, &c. Probably identical with *P. bulbaliseptica* (Oreste and Armanni, 1887), the bacillus of Barbone Disease.

History.

The disease was first described by Bollinger (1878), and the causative organism isolated by Kitt (1885).

Pathogenicity.

The organism is pathogenic in varying degrees for horses, cattle, sheep, pigs and the usual laboratory animals, but birds are more resistant.

Pathogenic Action.

In the acute form of the disease the incubation period is short and the mortality may reach 90 per cent. In acute cases widespread œdemas and hæmorrhages in the submucous, subserous and connective tissues and the

lymph glands are apparent ; in more chronic cases the lungs may be more particularly involved, presenting the signs of pleuro-pneumonia, or the disease may be of an enteritic nature.

Diagnosis.

The bipolar bacillus is easily isolated from the blood and organs in all the more acute forms of the disease.

Immunization.

Oreste and Armanni (1887) used pigeon passage to attenuate the virulence of *P. boviseptica*, and following Pasteur's prescription, inoculated buffaloes with infected pigeons' blood, thus conferring, they averred, active immunity upon the beasts. Lignières (1902) found oxen to be immunized against *P. boviseptica* after two inoculations of his multivalent attenuated living vaccine, which contained strains of all types. Hardenbergh and Boerner (1917) also used a living vaccine, choosing a strain of such virulence that it would kill rabbits and guinea-pigs, but not sheep or calves. They realized the danger attendant on such living vaccines, but considered them so much superior to killed ones as to make it worth while running the risk. Washburn (1918) protected rabbits against 4 m.l.d. of *P. boviseptica* by a killed *boviseptica* vaccine. Sheather (1918) in India found vaccine treatment was very useful where the pasteurellosis was enzootic. It should be given at the beginning of the season when the disease is most prevalent. D'Herelle and Le Louet (1921) reported that buffalo calves inoculated with living attenuated *P. boviseptica* suffered very slight reactions and were later shown to be immune. Attenuation of the vaccine strain was achieved by passing through rabbits and then growing in rabbit broth ; if grown in beef broth the strain was virulent.

Doyle (1923) injected 0·5 c.cm. of similar rabbit-broth culture into four Cyprus bulls, three of which had previously been injected with 170 c.cm. immune serum and 0·1 c.cm. of broth culture ; only one animal (one of the three injected ones) withstood the inoculation well ; two died and one was very ill. Rabbit passage and growth in rabbit broth had not attenuated the virulence of this strain.

D'Herelle (1921) was able to isolate bacteriophage from the dejecta of all buffaloes that had remained healthy in the midst of an epizootic of *boviseptica* infection, but rarely succeeded in doing so from animals in uninfected regions. He (1926) stated that even large 20 c.cm. doses of bacteriophage had no ill effect on the animals. Such big doses take 40 to 60 days in which to immunize ; small doses of 0·04 c.cm. do so in four days. He reported Le Louet as demonstrating a 66 per cent. immunity against 500 m.l.d. in 15 animals, 14 months after inoculation with the bacteriophage.

Gochenour (1924) and Buckley and Gochenour (1924) considered living vaccines too dangerous, even though attenuated strains are chosen. They used killed bacterial cultures or extract and filtered animal exudates, and protected animals against 500 m.l.d. Bennett (1926) also preferred killed vaccines for the same reason, i.e. living ones are too dangerous.

It is difficult to form an idea as to the real prophylactic value of vaccination against bovine pasteurellosis. Most opinions on the subject are the result of field experiments without adequate control by the observation of a sufficient number of uninoculated animals exposed to similar risks of infection. This fact is of particular importance, as outbreaks vary in severity to a marked degree, and usually end suddenly of their own accord. In this way a false impression may often be given that treatment by vaccination has lowered the morbidity rate and brought the epizootic to a standstill.

In laboratory work the evidence is conflicting. Doyle (1923), for instance, repeating in England the experiments of D'Herelle and Le Louet, was unable to use a method for protecting bulls which, the French workers reported, was capable in Cochin China of immunizing buffalo calves against as much as 100 m.l.d. The claim of the same experimenters to have immunized by inoculating with bacteriophage has not been confirmed.

Passive immunity. The serum of immunized animals is very largely used, and approved, as reported by Holmes (1910–11). He found that immediate protection lasting for four weeks is established in not less than 90 per cent. of the animals by doses varying from 5 to 20 c.cm. in cattle and 20 c.cm. and more in buffaloes. The method continues to be made use of in India, as reported by Sheather (1918) and Bennett (1926).

Pasteurella vituliseptica.

Synonym. B. vitulicidum.

The bacillus of septic pneumonia of calves.

Poels (1886) isolated the organism for the first time from an outbreak of septic pleuro-pneumonia in calves occurring in the neighbourhood of Rotterdam. It resembles the other members of the pasteurella group culturally and morphologically. It is probably identical with *P. bovi-septica*, which it closely resembles in its pathogenicity and immunology. There seems no good reason for keeping the two organisms apart. A list of the earlier papers devoted to this pasteurellosis and its bacterial agent is given by Schirop (1908).

Pasteurella oviseptica.

Synonym. P. ovis.

The bacillus of pneumo-enteritis of sheep.

The disease caused by this organism was investigated by Galtier (1889) in France and by Lignières (1898) in the Argentine. Later reports are by Raebiger *et al.* (1913), Newsom and Cross (1923), Marsh (1923), Hadley (1924) and Gerlach and Michalka (1925).

The organism occasions symptoms and lesions similar to those seen in the cattle pasteurelloses. Haan (1920) saw pigs develop pleuro-pneumonia after being let into a stall which had previously contained sheep ill with pasteurellosis. He considered that the two diseases must be identical, but Miessner and Schern (1910) could demonstrate no serological or

immunological relationship between *P. oviseptica* and *P. suiseptica* or any of the other pasteurella types. Lignières (1902) found his attenuated multivalent living vaccine effective; to produce immunity in sheep he inoculated 0·25 c.cm. of his two broth cultures in succession. Miessner and Schern (1910) tried Lignière's method; they gave 5 or 10 c.cm. of a 24 hour *P. oviseptica* broth culture kept at 42·5° C. for 5 days, followed a fortnight later by a 1 c.cm. dose of a similar culture that had stood at 42·5° C. for only 2 days. The lambs survived a test dose of 7 loops, which killed controls in 4 and 9 days, but their condition did not remain good, and on killing, 4 months later, lesions and living *P. oviseptica* were found in the organs. These authors also tried formalinized emulsions of *P. oviseptica*; 20 c.cm. were inoculated into lambs and a test dose of 2 loops was given intravenously; of 3 lambs, 1 died the following day, and 2 lived and were healthy when killed one month later; the controls died in 3 and 4 days. Agar growth emulsions and broth cultures were killed with formalin and shaken with beads; the centrifuged deposit protected 2 out of 4 lambs, the control dying in 1 day; the filtered supernatants did not protect.

Passive immunity was demonstrated by the same authors on rabbits; 4 animals were given 3 c.cm. of anti-*oviseptica* horse serum, followed after 1 or 3 days by 1/1,000 loop as test dose; 1 died, like the control in 1 day; the other 3 survived. They protected against a larger test dose of 1/100 loop by giving a second dose of immune serum after the test dose, both rabbits surviving. A combination of active and passive immunity was also tested; 4 lambs received serum together with bacterial extract: 2 died of the treatment, but the remaining 2 were seen, 20 to 34 days later, to be immune to a test dose of 2 loops of *P. oviseptica* which killed the controls in 24 hours.

Raebiger *et al.* (1913) confirmed Miessner and Schern's results. Later Raebiger *et al.* (1915) and Raebiger (1916) found that an immune serum alone was sufficient to lower mortality and prevent the spread of the disease to other flocks.

Pasteurella lepiseptica.

Synonym. P. cuniculi.

The bacillus of rabbit septicæmia and snuffles or influenzal catarrh.

This bacillus was first described by Gaffky (1881), and among the earlier investigators were Smith (1887) and Thoinot and Masselin (1889). It was later studied by Raebiger (1912), McCartney and Olitsky (1923), Tanaka (1926) and Smith (1927). It has been made an object of close study by Webster and his fellow workers. In a series of papers appearing from the year 1924 onwards in the *Journal of Experimental Medicine* the relationship of this organism to respiratory disease in the rabbit and its association with *B. bronchisepticus* have been established. This last Bull and McKee (1927) confirmed. Smith and Webster, (1925) have recorded cases of otitis media due to *P. lepiseptica*. Tanaka (1926) and Smith (1927)

have described abscesses varying in size from a grape to a walnut which lie free from the skin in the loose subcutaneous tissue; they may occur after either natural or experimental infection with *P. lepiseptica*. De Kruif (1921[1&2], 1922[1&2]) described a variant form, G, distinguishable from the normal, D, by its growth in broth as a granular sedimentation as opposed to a diffused turbidity. The D form is virulent, the G avirulent. While the two forms differ in their acid agglutinability, the optimum pH for G being 4·7 to 4·0 and that for D being 3·5 to 3·0, immunity and absorption experiments indicate antigenic agreement. Webster (1925) has shown that vaseline-sealed broth cultures or those to which small quantities of blood have been added are unfavourable to the conversion of the D variant into the G. Webster and Baudisch (1925) were able to demonstrate that certain inorganic iron substances of known chemical structure, e.g. Fe_3O_4 and Fe_2O_3, possess the same power as blood to prevent the D form changing to the G form; these substances are all oxygen-absorbing and have peroxidase properties.

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CHAPTER VII. *B. PSEUDOTUBERCULOSIS RODENTIUM* PREISZ.

By H. Schütze (Lister Institute, London).

The first experiments with this bacterium were probably those of Malassez and Vignal (1883), who inoculated guinea-pigs with material from a subcutaneous nodule in the arm of a child dead of tuberculous meningitis, and described the resultant infection as 'tuberculose zoogléique', because of the modified resemblance to the tuberculosis produced by Koch's bacillus; these workers were probably dealing with a double infection which was the occasion of the atypical zoogleic appearances. Under various names, Nocard (1885), Eberth (1886), Dor (1888), Charrin and Roger (1888), Pfeiffer (1889), Gratcher and Ledoux-Lebard (1888) and Zagari (1890) described, in a variety of animals, infections which seemed identical with that of Malassez and Vignal and due to the same micro-organism. It remained for Preisz (1894) to suggest for this microbe its present specific name, *pseudotuberculosis rodentium*, though, on account of its tendency under certain circumstances to grow in chains, he called it a *Streptobacillus*.

Characters of the Bacterium.

The organism varies in shape and size, according to the conditions of growth, from a stout, almost coccal form, under 1μ in length, to a definite rod with rounded ends, 2μ long. In liquid media there is a tendency to form chains and filaments. It is Gram-negative. Bipolar staining is frequently noticeable. Neither capsules nor spores are formed.

Though occasional workers (Courmont, 1889; Nocard and Masselin, 1889; Preisz, 1894; Klein, 1899–1900; Kossel and Overbeck, 1901; Byloff, 1906; Henschen, 1918) reported motility or the presence of flagella in strains of which some, at least, were undoubtedly *B. pseudotuberculosis rodentium*, the organism has generally been considered non-motile and lacking in flagella. Recent observers have, however, confirmed the veracity of these earlier statements. Klein (1921) noticed motility in one strain; Plasaj (1921) reported the existence of one extrapolar flagellum, though its demonstration was not always possible; Arkwright (1927) was able, like Kossel and Overbeck, to show that '*B. pseudotuberculosis rodentium* is motile when cultivated at 18 to 26° C., but not as a rule when grown at 37° C.'; he also demonstrated the existence of a flagellar antigen which is heat-labile and agglutinable in a flocculent manner, together with a heat-stable somatic antigen agglutinating in granular fashion.

Growth takes place readily on all ordinary media; according to Kakehi (1915–16), it is completely inhibited by strict anaerobiosis. The viability

of cultures is normally one of months. Growth in broth is diffuse in the case of the smooth variant ; later ring and pellicle formation occurs and a deposit forms ; the rough variant grows in clumped masses with a more marked pellicle and deposit, and possibly no turbidity at all. On agar the growth of the smooth variant varies with the temperature ; at 37° C. it is thin and dry, at 18 to 26° C. moist and slimy ; colonies are of an opaque, dirty white, and varying degrees of density ; occasionally interior veinings and spreading margins are to be observed ; the rough variant colony is denser, drier and more irregularly edged. On gelatin similar development occurs. No liquefaction takes place. Phosphatic crystals can be observed.

Milk is not coagulated, but is gradually rendered alkaline. Growth on potato is light brown in colour. Nitrates are not reduced. No indole or H_2S is formed. Exotoxins have been described by Dessy (1925) and Meyer (1928).

Sugars and alcohols, when attacked, are fermented without production of gas and there is no disagreement amongst workers (MacConkey, 1908 ; Saisawa, 1913 ; Kakehi, 1915–16 ; Lerche, 1926 ; Poppe, 1927 ; Meyer, 1928 ; Haupt, 1928 ; Gaté and Billa, 1928 ; Beck, 1928 ; Schütze, 1928) with regard to the following list of reactions. Acid in arabinose, arbutin, galactose, dextrose, glycerol, iso-dulcitol, lævulose, maltose, mannitol, rhamnose, trehalose, xylose. No acid in amygdalin, dulcitol, erythritol, inositol, inulin, lactose, raffinose, saccharose, sorbitol. But Roemisch (1921) reported one strain and Zlatogorov and Mogilevskaia (1928) a rough variant as acidifying dulcitol. It is advisable to test the fermentation properties at a lower temperature such as 26° C., as well as at 37° C., some strains apparently preferring the one temperature to the other, and to extend the time of observation to at least two weeks. Gaté and Billa (1928) obtained fermentation of only one sugar, lævulose, with their strain when freshly isolated from a guinea-pig epidemic. After several subculturings on artificial media it fermented all the usual substances except salicin. Rough variants, according to Zlatogorov and Mogilevskaia (1928) occasion fermentation more rapidly than do the corresponding smooth forms. In adonitol, Haupt, Kakehi, Poppe and Schütze report acid, MacConkey, no acid ; in dextrose, Poppe, Saisawa and Schütze report no acid, MacConkey and Lerche, acid ; in salicin, Haupt and Schütze report acid, Gaté and Billa, no acid.

The resistance of the bacterium to low temperatures is great, to raised temperatures such as 56° C. and higher, and to dessication, slight.

The organism appears to be widespread in nature and has been recovered from specimens of soil (Grancher and Ledoux-Lebard, 1889), dust (Chantemesse, 1887), water (Klein, 1889–1900), fodder (Lignières, 1898), and milk (Parietti, 1890).

Serology.

Agglutination by specific immune serum was demonstrated by Saisawa (1913) and Roman (1916) both of whom found that heterologous strains

agglutinated to a considerably lower titre than did the homologous strain. Roemisch (1921) inoculating guinea-pigs with three different strains, obtained good cross-agglutination with the three strains ; cross-absorption was possible in the case of two of the sera, results with the third being puzzling. Lerche (1927), examining 50 strains isolated from a variety of animals, obtained but small differences between the homologous and heterologous agglutination titres ; he was also able to show, by means of the absorption test, that all his strains were antigenically identical. Schütze (1928) demonstrated by agglutination tests the existence of three groups, depending on the constitution of their O antigens for differentiation, the H antigen being the same throughout ; by absorption tests he was able to show that two of these groups could each be split into two sub-groups or absorption types.

Complement fixation was demonstrated by Saisawa (1913) without any notable differentiation of strains ; Roman (1916), working with one serum and three strains found complement fixation practically confined to the homologous strain ; Bachman (1921) demonstrated the presence of complement-fixing bodies in guinea-pig serum eight days after infection. Zlatogorov and Mogilevskaia (1928) obtained complement fixation with smooth strains but not with rough.

Opsonin production by inoculation of killed and living cultures, was studied by Noon (1909), who found that the subcutaneous route gave a more rapid response than either the intravenous or intraperitoneal.

Precipitin reactions were reported by Zlatogorov (1904), using filtrates of 3 to 4 month's old cultures, but could not be demonstrated by Saisawa (1913), using filtrates only 2 weeks old and sera with low titres in agglutination and complement-fixation tests.

Intracutaneous reactions were successfully evoked by Bachman (1921) in infected guinea-pigs using a heat-killed saline suspension of agar-grown organisms ; also by Dessy (1925).

Cross-agglutinations with bacteria of alien species have been recorded by a number of observers ; they have, until recently, only concerned the very closely related microbe *B. pestis*. Zlatogorov (1904) agglutinated pseudotuberculosis strains with plague sera almost to titre limit ; Albrecht (1910) and Lerche (1927) found similar cross-agglutination, while Saisawa (1913) did not find any. Arkwright (1927) attempted cross-agglutination in the opposite direction and succeeded in agglutinating plague bacilli with pseudotuberculosis sera to $\frac{1}{10}$–$\frac{1}{4}$ of their specific titres. Zlatogorov and Mogilevskaia (1928), on the other hand, could not agglutinate plague with the three pseudotuberculosis sera with which they worked. Klein (1921) reported cross-agglutination between one pseudotuberculosis strain and paratyphoid B serum, and, though it is not quite clear from the data given that the strain was a genuine *B. pseudotuberculosis rodentium*, it is probably the same phenomenon as was reported by Schütze (1928), who discovered an agglutination affinity between one of his three groups of *B. pseudotuberculosis rodentium* and certain

members of the Salmonella group, viz.: Types—Schottmüller, Aertrycke, Stanley, Abortus Equinus, Derby and Reading, the relationship being confined to the O antigen.

Cross-precipitation experiments with *B. pestis* and *B. pseudotuberculosis rodentium* were carried out by Zlatogorov (1904) and MacConkey (1908), the former could get no cross-precipitation, the latter demonstrated quantitative differences, pestis filtrate giving in plague serum a more ready and more abundant precipitate than did pseudotuberculosis filtrate.

Cross-complement-fixation was attempted by Damperoff (1910); he obtained no deviation of complement by *B. pseudotuberculosis rodentium* and a plague serum.

Immunity.

Active immunity was conferred on guinea-pigs by Saisawa (1913) and Saceghem (1916), both using heat killed cultures; this Dessy (1925) was not able to achieve; he even demonstrated hypersusceptibility in rabbits which had previously been injected with the killed bacillus; the hypersusceptibility could not be transferred passively to other animals. Zlatogorov and Mogilevskaia (1927) derived from old broth cultures variant strains which were antigenically so altered that they no longer gave protection by immunization against infection with the parent strain.

Passive immunity could not be demonstrated in guinea-pigs by either Saisawa (1913) or Dessy (1925).

Active cross-immunity for *B. pestis* has been shown to exist by both MacConkey (1908) and Rowland (1912). The former immunized 15 guinea-pigs with killed, followed by living, inoculations, and showed their complete immunity to a dose of living *B. pestis* that killed 70 per cent. of the 10 controls; on another occasion he protected 88·5 per cent. of his 14 guinea-pigs against a dose that killed 85·7 per cent. of the 7 controls, the test dose being given nearly 7 months after the last immunizing dose. Filtered autolysates of *B. pseudotuberculosis rodentium* were seen to confer a similar immunity, in contradistinction to autolysates prepared from *B. pestis*, which only protected 28·5 per cent. of the guinea-pigs employed. Rats inoculated with living pseudotuberculosis cultures were seen after 5 months to possess considerable immunity for *B. pestis*; of the 13 tested, 8 survived, while all 5 controls died of the test dose. Rowland (1912), who, however, used a chloroform-killed vaccine or a water-soluble nucleoprotein derived from *B. pseudotuberculosis rodentium*, did not achieve any noteworthy immunity against *B. pestis* in rats. In guinea-pigs the chloroform-killed vaccine established a solid immunity, but the nucleoprotein was quite ineffective. His explanation of these differences is that for a vaccine to be efficient it must possess toxicity for the animal employed; pseudotuberculosis nucleoprotein is atoxic for both rat and guinea-pig, and will, therefore, protect neither; the whole bacterium is toxic for the guinea-pig but not for the rat; it, therefore, immunizes the former animal and not the latter.

Passive cross-immunity could not be demonstrated by Zlatogorov (1904) ; he found that guinea-pigs protected against *B. pestis* by injection with antiplague serum were not immune to *B. pseudotuberculosis rodentium*.

Differential Diagnosis.

The bacteria to which *B. pseudotuberculosis rodentium* seems most nearly related are *B. pestis* and the Pasteurella group. While a number of features distinguish it from the latter, from *B. pestis* differentiation may be a matter of great difficulty. *B. pseudotuberculosis rodentium* grows more rapidly and more luxuriantly on artificial media than does *B. pestis* ; these characters, like so many others to be mentioned below, are, however, hardly definitive. No serological or immunological system of differentiation has, as yet, been worked out, though with the more complete knowledge of pseudotuberculosis agglutination now available, this should be possible.

A number of media have been devised, of which none is generally accepted as absolutely diagnostic. The following statements are those made by the authors of the media :

(1) Hankin and Leumann's (1897) 3 per cent. salt agar occasions involution forms in *B. pestis*, but not in *B. pseudotuberculosis rodentium*. (2) Skorodumoff and Somorowitsch's (1926) 6 to 10 per cent. salt agar suppresses the growth of *B. pseudotuberculosis rodentium*, but not that of *B. pestis*. (3) Petrie and Macalister's (1910–11) malachite-green broth is decolorized rapidly by *B. pseudotuberculosis rodentium*, but only to a slight extent and often quite slowly by *B. pestis*. (4) Otten's (1926) glucose peptone water is made alkaline by *B. pseudotuberculosis rodentium* and not by *B. pestis*. (5) Himmelfarb's (1927) maltose peptone water is fermented more rapidly by *B. pseudotuberculosis rodentium* than by *B. pestis*. (6) Nikanorov's (1927) thymol-blue sugar-free broth serves as indicator for the greater alkalinization which results from *B. pseudotuberculosis rodentium* than from *B. pestis*.

The following table gives the chief differentiating characters of these three closely related bacteria :

Bacterium.	Acid Production in :				Indole and H_2S.	Motility.	Litmus Milk.	Virulence for White Rat.	Growth on Bile Salt Media.
	Glycerol.	Rhamnose.	Saccharose.	Sorbitol.					
B. pseudotuberculosis rodentium ..						*	alkaline*		
B. pestis	±	+	—	—	—	—	neutral	+ *	+
Pasteurella ..	—	—	+	+	—	—	neutral	±	—

* See text for exceptions.

It will be seen that, whereas *B. pseudotuberculosis rodentium* and Pasteurella are distinguishable by a number of features, *B. pseudotuberculosis* and *B. pestis* are only differentiated in this table by three characteristics, all of which may be subject to modification : (1) Motility—while this is a very valuable diagnostic character for *B. pseudotuberculosis*, non-motile, O forms must occur in this as in other motile species. (2) Alkalinity in litmus milk—as poor alkali producers are reported by Nikanorov (1927) such pseudotuberculosis strains may be missed by this test, as well as by those special media mentioned above which depend on production of alkali for their differentiating powers. Zlatogorov and Mogilevskaia (1928) have found a deficiency of alkali production associated with smoothness ; it is, according to them, particularly the smooth variant which is culturally so akin to *B. pestis*. (3) Virulence for the white rat—freshly isolated strains of *B. pestis* may be expected to have retained their virulence, but older strains may have lost it.

Failing the presence of motility, the differentiation of *B. pseudotuberculosis* from *B. pestis* must be decided by the cumulative evidence of a number of tests, the most important of which, for recent strains, no doubt, being the virulence test, and after that, probably, the production of alkalinity.

A non-gas-producing Salmonella is a possible source of confusion, so many of its biochemical reactions being identical with those of *B. pseudotuberculosis rodentium ;* Schütze (1928) has also pointed out the existence of cross-agglutinations between certain types of *B. pseudotuberculosis rodentium* and certain Salmonella types ; but in spite of these relationships, examination by agglutination, and, if necessary, absorption should effect differentiation, and the presence or absence of motility at 37° C. be an aid thereto.

Pathogenicity.

A great variety of animals are susceptible to the disease ; it has been described in the horse (Schlaffke, 1921), cow (Mazzini, 1897), pig (Sčennikov, 1928), goat (Baumann, 1927), rabbit (Roemisch, 1921), hare (Lerche, 1927), cat (Leblois, 1920), guinea-pig (Ramon, 1914), wild rat (Meyer and Batchelder, 1926), hen (Christensen, 1927), turkey (Lerche, 1927), pigeon (Beck, 1928), canary (Heelsbergen, 1927), monkey (Christiansen, 1918), and man ; many of these species are rarely attacked and individual strains are found to have individual preferences, reminding one of the varying pathogenic affinities displayed by different Pasteurella strains.

In Man. A certain number of human cases have been reported, the earliest description of the disease, indeed, being Malassez and Vignal's (1883) infection of guinea-pigs with material from a subcutaneous nodule in the arm of a child, though the possibility of the disease having been pre-existent in the animals has to be considered. Several cases are left in doubt owing to the meagre descriptions supplied by the authors, among

these being Manfredi's (1886) two cases of pneumonia, Hayem's (1891) case of gastro-enteritis, Mazza and Mensi's (1896) broncho-pneumonia with purulent pleurisy, Courmont's (1897) arthritis, Weltmann and Fischer's (1914) otitis media, and Bayer and Herrenschwand's (1919) conjunctivitis.

It is noteworthy that the four indubitable cases of infection in man were all abdominal in type. Albrecht's (1910) was one of acute appendicitis ; he established the ætiology, not indeed, by direct culture, but by inoculation of material from the resected gut into two guinea-pigs, only one of which developed the disease. Lorey (1911) isolated the bacterium from the blood during life and from the spleen, liver, gall-bladder, spinal cord and blood after death, of a man whose symptoms, fever, diarrhœa, &c., suggested typhoid. Saisawa (1913) also succeeded in recovering the organism from the blood during life of a man with high temperature and intestinal symptoms. Roman (1916) isolated it from the liver and spleen of a case of pyrexia accompanied by abdominal pains.

Pathogenic Action.

Natural infection probably takes place in most cases through the alimentary canal ; the lesions are very largely confined to the abdominal cavity. A notable feature is the involvement of the mesenteric glands, which may become very large and confluent and develop caseation ; nodules are formed in the follicles of the intestine ; the liver and spleen can be thickly studded with similar whitish nodules of varying size. A picture resembling genuine tuberculosis is thus presented ; a similar appearance may, however, be occasioned by other bacteria, in particular the Salmonella, which has, no doubt, been the frequent occasion of confusion. Further nodules may be present throughout the peritoneal cavity and occasionally in the lungs and pleuræ. The content of these nodules is of a smooth thick creamy nature ; calcification does not occur and epithelioidal and giant cells are generally considered to be absent, all points of differentiation from the histology of plague.

Ramon (1914) has described three types of disease in the guinea-pig : (1) An acute septicæmic form with death in 1 to 2 days and few macroscopic post-mortem signs ; (2) the classical form with pronounced abdominal features accompanied by diarrhœa and emaciation and death in 3 to 4 weeks, (3) a glandular form involving the sublingual and cervical regions.

Zlatogorov and Mogilevskaia (1928) investigated the virulence of rough and smooth forms derived from two different strains ; in one case both variants were about equally virulent, in the other, the smooth was more virulent than the rough.

The bacillus is usually isolated on ordinary media without difficulty, and in pure culture, from the blood at death, or from the lesions.

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